

UNIVERSITY OF BRISTOL



THE ANNUAL REPORT

OF THE

Agricultural and Horticultural
Research Station

(THE NATIONAL FRUIT AND CIDER INSTITUTE)

LONG ASHTON, BRISTOL

1960

BATH :

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Beatrice Alice Crang.



Stanley Herbert Bennett.

In Memoriam.

BEATRICE ALICE CRANG.

Miss Crang, after graduating from Bedford College in 1930, held the post of Food Chemist in Messrs. Lyons' Research Laboratories until her appointment at Long Ashton in 1938.

Within five years of her appointment she had expanded the staff of the Domestic Food Preservation Section by the addition of Miss Marjorie Jones (now Mrs. Croxall), Miss Dilys Jones (now Mrs. James) and Miss Sturdy (now Mrs. Alvis). With this increased staff, and her own store of experience, Miss Crang rapidly broadened the research programme, particularly in microbiology, and at the same time intensified the Section's advisory services. These were greatly in demand during the war years, when they included the organization and regulation of jam-making at rural centres, the use of Campden preserving tablets, the domestic extraction of sugar from sugar-beet and the production of tomato juice. Miss Crang's "Preserves for all Occasions", published in 1944 as a specially-commissioned Penguin Handbook and now in its fourth edition, has become the standard popular reference book. The author gave the royalties from this publication during the war years to Red Cross funds.

Meantime, on the research side, studies of the ascorbic acid and riboflavin content of processed vegetables were made in 1942-46, and a number of papers on the blackening of cooked potatoes were published towards the end of the war. The enzymic browning of bottled pears was examined in 1948 and the inactivation of the causative oxidases, in plums and apples as well as pears, was studied in the following year.

In 1950 Miss Crang started the systematic investigation of the processing qualities of established and new varieties of fruits and vegetables; the merits and demerits of each kind for bottling, canning and deep-freezing were critically assessed. This work has proved of great value, not only to the housewife but also to commercial processors. At the same time, fundamental studies were made of the temperature changes taking place in the container during the heating and cooling sequence in canning and bottling, and these led to revisions of the processing times and temperatures officially recommended in the Ministry of Agriculture Bulletin No. 21, "Domestic Preservation of Fruit and Vegetables". This bulletin, of which Miss Crang became after 1939 the principal author, is now in its ninth edition.

A new phase of Miss Crang's work began in 1953, when it became apparent that no trustworthy standards for the home preservation of meat and poultry products existed in this country. The work on this problem culminated in the publication in 1958 of an official bulletin entitled "Domestic Preservation of Meat and Poultry". Separate papers were issued on the domestic freezing of pork and pork products and on the storage of frozen bacon, brawn and venison.

Miss Crang's gift of clear exposition brought continual requests for her services as a lecturer. Her fame led to invitations to Norway, Denmark, Germany, Switzerland and the United States, and her courses of instruction were attended by people from many countries.

Her contributions to Long Ashton were by no means confined to her work for her Section. Her great personal charm, equanimity of outlook and the gift for sharing in the interests and enjoyment of others endeared her to all.

Miss Crang died at the age of 52 on 1st April, 1960. At the memorial

service in St. Paul's Church, Bristol, on 9th April, the esteem in which she was held was demonstrated by the presence of so many members of the Long Ashton and University staffs, representatives of the National Federation of Women's Institutes, the Women's Voluntary Services, of County authorities and Colleges of Domestic Science, the Association of Home Economists and the National Federation of Young Farmers' Clubs, of the Ministry of Agriculture and of the Bristol Sorooptimists, of which she was a Past President. All paid tribute to a life of unstinting service, grievously cut short in full career.

STANLEY HERBERT BENNETT.

Stanley Herbert Bennett, born in 1916, was educated at Lydney Grammar School and the University of Bristol, from which in 1938 he graduated B.Sc., with Honours in Zoology, and was awarded the Diploma in Education in 1939. He then took part in an investigation on methods of mole destruction at Long Ashton. In the latter part of 1940 he was appointed Assistant Advisory Entomologist, and from 1941 to 1946 he was seconded as Advisory Entomologist to the Long Ashton Field Station at Evesham. In 1946 he came back to Long Ashton as Research Entomologist.

Dr. Bennett's work followed three major paths. First came his researches on insect biology and ecology, which continued throughout his career. This interest was exemplified by papers on the flight period of the codling moth in the West Country, his observations on fruit aphids, sawflies and midges, and his more recent work on the insect populations of fruit trees, particularly the leaf hoppers. Secondly, Dr. Bennett worked extensively on the field control of insect pests by spraying, evaluating the merits of DNC and the organic thiocyanates. From 1953 he concentrated on an intensive study of the systemic phosphorus insecticides and, in collaboration with Dr. W. D. E. Thomas and Mr. R. F. Batt, published a series of authoritative papers on the absorption, translocation and breakdown of these compounds in plants. His distinction in this field was recognised by the fact that he was commissioned in 1957 to review the behaviour of systemic insecticides for the Annual Review of Entomology.

Dr. Bennett was appointed to the Plant Pests and Diseases Committee of the Association of Applied Biologists in 1947 and elected Secretary of the Committee for 1948-49. His term of office was signalised by his organisation of a highly successful conference on spray damage, held at Imperial College in January, 1949. His services as an adviser to the fruit-growing industry were equally noteworthy; his entomological knowledge was combined with a highly practical outlook and his advice to growers during the war years was widely valued.

With his colleagues at Long Ashton "Ben" was universally popular. His outstanding characteristics were a warm-hearted impulsiveness and a gift of sympathy and understanding which was particularly in evidence in his relations with junior members of staff. He played hockey for Gloucestershire and he was a tower of strength in the Tennis Club. When the Recreational Club was formed at Long Ashton in 1957 he was the obvious choice for Chairman and he held that office at the time of his death.

On 29th May, 1960, he died suddenly from a heart attack after spraying in his own orchard at Tickenham. The complete unexpectedness of this blow stunned and dismayed his many friends and colleagues, whose profound sympathies go to Mrs. Bennett and his son and daughter in their tragic loss.

INTRODUCTION.

By H. G. H. KEARNS, Director.

The Station has suffered this year the loss by death of two senior members of staff, Miss Alice Crang and Dr. Stanley Bennett, whose obituary notices precede this Introduction.

We also record with regret the death on 16th July of the Honourable James W. Best, who had been a member of the National Fruit and Cider Institute since 1932. He represented the Dorset Federation of Farm Cider Makers on the Governing Body of the Institute from 1933 to 1940, and since 1953 had represented the Bath and West Society.

Sir William Slater, K.B.E., F.R.S., Secretary of the Agricultural Research Council since 1949, retired at the end of June. He was succeeded by Professor E. G. Cox, T.D., D.Sc., F.Inst.P., F.R.I.C., F.R.S., who had previously held the Chair of Inorganic and Structural Chemistry in the University of Leeds.

Mr. Morley Davies, Senior Education and Advisory Officer (Science), Ministry of Agriculture, Fisheries and Food, retired at the end of last year, and was succeeded on 1st January by Dr. Norman Pizer.

Mr. Giles Toker was appointed by the Agricultural Committee of the University at their February Annual Meeting to membership of the Finance and Business Sub-Committee.

We record with much pleasure the award of C.B.E. in the Birthday Honours to Dr. F. R. Tubbs, Director of East Malling Research Station, and in the New Year Honours the award of O.B.E. to Mr. J. M. S. Potter, Director of the N.A.A.S. National Fruit Trials, who serves on the Governing Body of the National Fruit and Cider Institute as a representative of the Royal Horticultural Society. The election of Dr. R. L. Wain, Wye College, University of London, as a Fellow of the Royal Society in March, gave great pleasure to his friends at Long Ashton, where he worked from 1940 to 1945.

Co-operation with other research and advisory organizations has been maintained during the year. The Annual Refresher Course for Horticultural Officers of the National Agricultural Advisory Service was held at Long Ashton on 12th and 13th July and was attended by 41 N.A.A.S. officers and 11 officers of Local Education Authorities. The Horticultural Education Association held their annual conference in Bristol from 5th to 9th September and visited the Station on 7th September. Dr. Bould acted as Conference Chairman, Mr. Campbell as Secretary and Mr. Catlow as Treasurer; Professor Kearns and Professor Wallace presided over two of the lecture sessions. Dr. Morgan and Mr. Mapother contributed to another H.E.A. Conference, held at Pershore on 20/21st April, when they gave talks and demonstrations on biological requirements of spraying and the use of fluorescent tracers for the detection of spray deposits.

The Director has been co-opted on the Experiments and Education Committee of the Bath and West Society for the year 1960/61; he has been appointed Chairman of the newly-formed Scientific Advisory Committee of the Ministry's Agricultural Chemicals Approval Scheme, on which Dr. Martin is also serving.

Dr. Pollard has accepted an invitation to serve on the Horticultural Marketing Council, set up in May by the Ministry of Agriculture and the Secretary of State for Scotland, to work for the improvement of marketing of fruit, vegetables and flowers, and the development of trade in these commodities.

Mr. Clarke is assisting Bristol Siddeley Engines Ltd. with problems involved in the development of jet engine blades; at the request of Professor Heilbronn, he has given a lecture course on statistics for biologists in the Department of Mathematics in the University.

A short tour of fruit farms in Somerset and Devon was arranged by Mr. Hobbs for members of Station staff on 1st and 2nd June. Thanks are due to those fruit growers who made the tour so informative. The staff of the Pomology Section also paid an exchange visit to East Malling Research Station in September.

At the invitation of East Malling Research Station, Dr. Martin gave the 13th Amos Memorial Lecture at Wye College on 3rd November on "The plant cuticle: its structure and relation to spraying problems".

The regulations governing classes of membership and subscription rates of the National Fruit and Cider Institute were revised as from 1st January, 1960, and a brochure containing a short history of the Institute and setting out terms of membership is now issued to prospective members. In addition, a booklet containing lists of the Governors and Members was distributed during the year.

With the improved facilities now available in the Common Room it has been possible to invite visiting speakers to give staff talks during the winter months. Mr. Gordon A. Maclean of Kingston Bagpuize presented a colour film of his pillar system of apple production, Dr. L. Broadbent of the Glasshouse Crops Research Station spoke on "The spread and control of plant viruses", and Mr. L. N. Staniland, N.A.A.S., Bristol (with Mr. H. R. Mapother of the Research Station) explained the use of fluorescent tracers in the study of spray deposition.

Good use of the Common Room was also made during the visit of the Mass Radiography Unit to Long Ashton, when over a hundred members of staff and their families were given chest X-ray examination; this avoided the necessity for staff to travel into Bristol.

The A.P.V. Company Ltd. of Crawley, Sussex, have made a prolonged loan to the Station of a heat exchanger and ancillary equipment for the preparation of fruit juices, and the Kestner Evaporator & Engineering Co. Ltd. have also loaned a small-scale climbing film evaporator and volatile fraction recovery unit. The Station is greatly indebted to these companies for the loan of this valuable equipment.

Assistance has been provided during the year, through the funds of the National Fruit and Cider Institute, for the purchase of text books for junior staff who study at the College of Technology and use the Station library after hours. In addition, Chambers' Encyclopaedia has been bought with Institute funds.

The conditions of the award of The Fruiterers' Company Prize for the best essay on the fruit industry by members of the junior staff have been amended by the Company's Fruit Culture Council, who have agreed that a monetary award of £7 10s. shall be made each year in October to the student

in the Assistant (Scientific) grade or a member of the plantations staff who has made the best progress at the College of Technology or contributed outstanding work concerned with fruit culture to the Research Station. The first award was made to Miss Gillian North-Smith of the Pomology Section.

Staff.

The Station's first Domestic Supervisor, Miss Patricia Batcheller, resigned at the end of July to accept a post in Kenya, and was succeeded by Mrs. Joan Swatridge, a proved member of the Refectory staff.

Resignations :

Miss M. F. Roberts, B.A., M.Sc., Analytical Chemistry Section, from 30th November.

Miss E. M. Filer, Assistant Experimental Officer, Domestic Food Preservation Section, from 30th April, on her marriage : as Mrs. Burt she continued part-time duties until 30th September.

Miss A. E. Turner, Assistant (Scientific), Pomology Section, from 31st March.

Miss J. J. Bird, Assistant (Scientific), Cider and Fruit Juices Section, from 30th June.

Mr. D. J. Sissons, Assistant Experimental Officer, Cider and Fruit Juices Section, from 31st August.

Mrs. K. A. Stear (née Taylor), Assistant Experimental Officer, Plant Physiology Section, from 31st August. Mrs. Stear continued part-time duties until 31st December.

Miss G. J. Browning, Assistant (Scientific), Entomology Section, from 24th September.

Mr. W. K. Hancock, Assistant (Scientific), Nutrition of Fruit Plants Section, from 30th November.

Mr. A. Mountjoy, Laboratory Attendant, from 5th October.

Miss P. Curtis, Shorthand Typist, Grade I, from 31st August.

Miss P. R. Hale, Copy Typist, from 18th November.

Appointments :

Mr. R. Parfitt, Assistant (Scientific), Nutrition of Fruit Plants Section, from 1st January.

Mr. T. D. J. Rogers, Skilled Mechanic, Workshops, from 11th January.

Miss L. M. Lardner, Assistant (Scientific), Pomology Section, from 4th April.

Miss A. M. Newbery, Assistant (Scientific), Domestic Food Preservation Section, from 25th July.

Miss C. M. Howse, Assistant (Scientific), Cider and Fruit Juices Section, from 26th September.

Mr. W. A. Taylor, Assistant (Scientific), Nutrition of Fruit Plants Section, from 26th September.

Miss M. E. Tandy, Assistant (Scientific), Entomology Section, from 17th October.

Mr. O. G. Tucknott, Assistant (Scientific), Cider and Fruit Juices Section, from 1st November.

Miss S. J. Lamb, Junior Clerk, from 18th January.

Miss M. J. Hassett, Shorthand Typist, Grade I, from 10th October.

Miss D. M. Ivory, Cook, from September 5th.

National Service :

Mr. G. J. Dickes, Assistant Experimental Officer, Analytical and General Chemistry, called up for National Service from 25th July.

Visits Abroad :

Mr. H. R. Mapother visited North Borneo from 29th February to 29th March on the invitation of the Department of Agriculture, to investigate the spraying of cigar-wrapper tobacco to control Frogeye Disease. The cost of the visit was met jointly by the Department of Agriculture and the Darvel Tobacco Plantations Ltd.

Dr. L. C. Luckwill attended the Symposium on Virus Diseases of Fruit Trees, Copenhagen, from 25th to 30th July.

Dr. H. E. Davenport contributed a paper on " Macro-molecular structure and biological function " at the Joint Symposium of the International Unions of Biological Science and of Biochemistry, held in Stockholm from 12th to 17th September.

Mr. K. G. Stott carried out a survey of willow-growing in East and West Germany, Poland and Holland between 9th September and 19th October.

Mr. G. M. Clarke attended the Symposium of the Biometric Society on Quantitative Methods in Pharmacology, held at Leyden, Holland, from 10th to 13th May.

Dr. C. F. Timberlake spent a study period from 28th March to 25th June with Professor Schwarzenbach, Laboratorium für anorganische Chemie, Eidg. Technische Hochschule, Zurich, Switzerland.

Dr. D. J. D. Nicholas gave a paper at, and received a travel grant from, the 7th International Soil Science Congress held in Madison, Wisconsin, U.S.A., from 5th August to 14th September.

Mr. D. L. Abbott attended the Symposium on Fruit Tree Physiology, Dresden, East Germany, from 29th August to 2nd September.

Dr. W. D. E. Thomas attended the Congress on Surface Activity, Cologne, Germany, from 12th to 17th September.

Degrees, Honours, etc.

Mr. G. J. Dickes, Assistant Experimental Officer, Analytical and General Chemistry Section, was awarded the qualification of Grad.R.I.C. in May 1960.

Miss Gillian North-Smith, Assistant Experimental Officer, Pomology Section, as reported above, was awarded The Fruiterers' Company Prize for 1960.

Buildings and Land.

The Refectory was completed at the beginning of the year and provides a five-day service of lunch and tea for the staff. The well-equipped kitchen, dining room and a comfortable Common Room in pleasant, colourful surroundings, have provided greatly improved conditions for meals and for entertaining the many visitors to the Station. The building will be invaluable for regular

functions during the Station's year, including the meetings of the National Fruit and Cider Institute, conferences, lectures, staff meetings, etc. The Common Room is equipped for slide and film projection. The building provides facilities for various evening social functions arranged by the Recreational Club, including the Christmas Party, dances, folk dancing, table tennis, etc.

Now that the Refectory is completed, the canteen has been converted back to a Library Annexe. The Library has been completely overhauled, redecorated and provided with light oak furniture, including stands for periodicals, a showcase for books of historic interest, librarian's reception desk, bookcases, refectory type tables and chairs. It now fulfils its proper function as a quiet place for study.

The old Main Laboratories have been completely rewired for electricity supply by the Station Workshop staff.

The Workshop staff have had an exceptionally heavy year of work arising out of the new buildings and facilities made available to the scientific staff. Items of particular interest include major adaptations in the Cider House to accommodate additional equipment. A small-scale Kestner climbing film evaporator and volatile fraction recovery unit has been erected in the centre of the building, after modifications to roof and floor; a fruit conveyor and elevator has been installed. Electrical, water and steam services and instrumentation have been installed for an A.P.V. heat exchanger. The steam and water supply services have also been re-arranged in the bottling room, and the performance of a vertical boiler improved.

Three covered concrete pits, each 78 ft. $\times$ 4 ft., with service paths and automatic watering, all enclosed in a bird-proof, nylon cage, have been constructed for the Nutrition of Fruit Plants Section.

Six concrete plunge beds, each 100 ft. $\times$ 4 ft., with pathways, have been completed for the Greenhouse/Laboratories. It is proposed to instal automatic watering in the near future.

The Header House of the Greenhouse/Laboratories has been fitted out with benching and cupboard units and services where necessary. Additional mercury-vapour irradiation lamps have been installed in several greenhouses. Six large nylon insect cages were installed in one half of the entomological greenhouse for investigations on Big Bud Mite of black currants and on Leaf Hoppers.

The heating of the engineering workshops has been converted to oil fuel. The wood workshops have been modified to accommodate additional wood-working power tools.

Open Days.

Cider Sampling Day was held on 5th May. Much of the programme was devoted to perries, following the extensive survey of varieties made in the 1959 season. The ciders shown illustrated the effects on cider quality of juice concentration and the retention of the volatile components of apple flavour. Demonstrations in the Cider House included illustrations of new continuous fruit presses and new plastic containers for cider. In the plantations visitors were shown methods of propagation of cider and perry varieties, virus testing and the use of heat therapy for virus-infected stocks.

The Annual Field Day for Members and the Annual General Meetings of the Members and the Governors were held on 28th July. Demonstrations in orchard plots included the establishment of bush perry pears in grass, the high working of plums against bacterial canker, the performance of the virus-tested loganberry LY59, residual herbicide trials and mineral deficiencies in raspberries.

At the Annual Meeting of the Institute, Captain A. Haggard and Mr. A. Woodall were elected Honorary Life Members, as a token of appreciation of their long service to the Station.

Visitors.

The number of parties visiting the Station was 54, compared with 45 in 1959. Visitors included the Biochemical Society, the Society of Chemical Industry and the Horticultural Education Association, who were all holding their Annual Meetings in Bristol, and a touring group from the Eighth International Grassland Conference, meeting in Reading.

Organized Parties, 1960.

Ashton Park School.
 Almondsbury Women's Institute.
 Association of Rural District Council Surveyors.
 Biochemical Society.
 Birmingham University Botany Department.
 Bristol Civic Society.
 Bristol and District Chelt Club.
 Bristol Soroptimists' Club.
 Bristol University Drama Department.
 Bristol University Ladies' Tea Club.
 Wm. Butler & Co. (Bristol) Ltd. : Advisory Staff.
 Careers Advisory Officers : S.W. Region.
 Chipping Sodbury Grammar School.
 Clifton High School for Girls.
 Colombo Plan Students.
 Domestic Food Preservation Course : Fruit and Vegetables (2).
 Downend Horticultural Society.
 Downend Secondary School : Young Farmers' Group.
 Efford Experimental Horticulture Station : Plantations Staff.
 Electrical Association for Women, Bridgwater.
 Exeter University Biological Society.
 Fisons Fertilizers Ltd. : Horticulture Advisory Section.
 Gardenhurst School, Burnham-on-Sea.
 Gloucester Technical College.
 Hambrook School Parent-Teachers Association.
 Henbury and Brentry Young Conservatives.
 Henbury County Secondary School.
 Hereford Training College.
 Horticultural Education Association.
 Institute of Electrical Engineers : Graduate Section.
 Institute of Bankers : Bristol Centre.
 VIII International Grassland Congress.
 May and Baker Ltd., Horticulture Advisory Officers.
 N.A.A.S. and L.E.A. Horticultural Officers.
 N.A.A.S. Fruit Group.
 Nottingham University Horticulture Students.
 Oakfield Electricity Sports and Social Club.
 St. Dunstan's Secondary School, Glastonbury.
 Scientific Sub-committee on Toxic Chemicals in Agriculture.
 Sexey's School, Bruton.

Society of Chemical Industry : Annual General Meeting.
 Society of Chemical Industry : Pesticides Group.
 Somerset Farm Institute : Horticulture Students.
 South West Electricity Board : Students' Society.
 Sunny Hill Girls' School, Bruton.
 Taunton and District Amateur Winemakers' Circle.
 Waveney Apple Growers Ltd., Suffolk.
 Westbury Young Conservatives.
 Whitchurch Women's Institute.
 Wills Hall Scientific Society.
 Wotton-under-Edge Secondary School : Rural Science Class.
 Wye College : Horticulture Students.
 Youth Hostels Association : Bristol Social Group.

Visitors from Overseas, 1960.

- Australia :* Mr. T. J. Beckmann, Queensland.
 Mr. M. V. Carter, Waite Institute, Adelaide.
 Dr. Eileen Fisher, Department of Agriculture, Victoria.
 Dr. A. Johnson, Boolaroo, N.S.W.
 Dr. Gordon B. Jones, C.S.I.R.O., Adelaide.
 Professor G. W. Leeper, University of Melbourne.
 Mr. R. Paddick, Tobacco Research Station, Victoria.
 Mr. J. Sedgwick, N.S.W.
- Bechuanaland :* Mr. I. J. Lewis, Tsetse Fly Control, Maun.
- Canada :* Professor and Mrs. Blythe Eagles, University of British Columbia.
 Dr. D. A. Chant, Vineland, Ontario.
 Dr. J. Craigie, Atlantic Research Laboratory, Halifax, Nova Scotia.
 Professor H. M. Good, Queen's University, Kingston, Ontario.
 Dr. C. H. Goulden, Department of Agriculture, Ottawa.
 Professor G. H. Harris, University of British Columbia.
 Mr. J. Matthews, Penticton, British Columbia.
 Col. S. C. Oland, Halifax, Nova Scotia.
 Dr. E. Zubecki, Vineland, Ontario.
- Chile :* Dr. J. F. Steel, Concepcion.
- Colombia :* Dr. Carlos Luna, Bogota.
- Costa Rica :* Mr. Gordon Havord, Turrialba.
- Denmark :* Mr. R. A. Jorgensen, Lyngby.
- Dominica :* Mr. A. E. Pugh, Colonial Development Corporation.
- Eire :* Mr. J. F. Connolly, Department of Agriculture, Dublin.
- Finland :* Dr. A. S. Varis, Tukkurila.
- France :* Mr. J. C. Malherbe, Calvados.
- Germany :* Mr. V. Hahn, Munich.
- Ghana :* Dr. D. Acquaye, W.A.C.R.I., Tafo.
 Mr. A. E. L. Buahin, Accra.
 Mr. R. J. Hurd, W.A.C.R.I., Tafo.
 Mr. P. D. Turner, W.A.C.R.I., Tafo.
- Holland :* Mr. P. M. Felius, Wilhelminadorp.
 Mr. C. H. Henkens, Gröningen.
 Mr. J. van Hennik, Wilhelminadorp.
 Mr. and Mrs. D. C. Post, Wageningen.
 Mr. H. Vos, Wageningen.
- Honduras :* Mr. R. H. Stover, La Lima.
- Hong Kong :* Dr. D. Y. Lin, United College.
 Mr. Y. C. Lin, Department of Agriculture.
- India :* Professor K. S. Bhargava, Gorakhpur University.
 Dr. A. Jha, Bihar.
 Mr. C. B. Rao, Andhra Pradesh.
 Dr. K. Singh, Punjab.
 Dr. L. Venkataratnam, New Delhi.
- Iran :* Dr. E. H. Hodyat, Teheran University.
- Israel :* Mr. H. Baumann, Daliah.
 Dr. Y. Gutter, Rehovot.
 Mr. E. Harman, Ministry of Agriculture.
 Mr. Z. W. Markus, Haifa.
 Mr. M. Shafrir, Mishmar Haemek.
 Mrs. A. Yaron, Haifa.
- Italy :* Professor E. Baldini, University of Bologna.

<i>Italy :</i>	Professor F. Scaramuzzi, University of Pisa.
<i>Japan :</i>	Dr. Tanaka, Tokyo.
<i>Kenya :</i>	Dr. E. M. Chenery, Tea Research Institute, Kericho. Mr. M. Graham, Department of Agriculture. Mr. B. Hanger, Ruiru. Dr. R. G. Poultney, Grassland Research Station, Kitale. Mr. D. H. Vell-Smith, Nakuru. Mr. P. E. Wheatley, Department of Agriculture.
<i>Lebanon :</i>	Mr. Robert Kfoury, Khanchara.
<i>Malaya :</i>	Mr. E. A. Rosenquist, Layang. Mr. J. M. Ross, Rubber Research Institute. Mr. W. P. Ting, Department of Agriculture.
<i>New Zealand :</i>	Mr. S. Frew, Department of Agriculture.
<i>Nigeria :</i>	Miss P. E. Adewale, Education Department, Lagos. Mr. R. A. Bull, W.A.I.F.O.R., Benin City. Mr. R. Leach, W.A.I.T.R., Kaduna. Mrs. T. A. Manuwa, Lagos. Dr. K. W. Smilde, W.A.I.F.O.R., Benin City. Mr. P. R. Tomlinson, Samaru, Northern Nigeria.
<i>North Borneo :</i>	Mr. A. Kingsley-Pallant, Mostyn Estates.
<i>Norway :</i>	Professor M. Edelen, Vollebakk.
<i>Poland :</i>	Professor M. Birecki, Warsaw. Mr. A. Scynczyk, Skierniewice. Mr. R. Kowalik, Warsaw. Mr. S. Mosinski, Warsaw. Mr. W. Oleksiak, Skierniewice.
<i>Portugal :</i>	Dr. H. Seabia, Lisbon.
<i>Rhodesia :</i>	Dr. C. H. Cronin, Fisons Pest Control Ltd., Rhodesia. Mr. G. B. Emslie, Lusaka, Northern Rhodesia. Mr. and Mrs. C. W. Lynn, Department of Agriculture, Northern Rhodesia. Dr. Iain McDonald, Tobacco Research Board. Mr. F. Puczykowski, Ministry of Agriculture.
<i>South Africa :</i>	Dr. R. A. Christ, Co-operative Citrus Exchange, Pretoria. Mr. J. Fisher, Johannesburg. Dr. J. W. Geyer, Pretoria. Mr. P. D. de Graaff, Cape Town. Dr. K. C. McOnie, Co-operative Citrus Exchange, Pretoria. Dr. L. J. Neethling, Shell Chemicals.
<i>Swaziland :</i>	Mr. J. F. Pryde, Colonial Development Corporation.
<i>Switzerland :</i>	Dr. and Mrs. R. Fritzsche, Wädenswil. Dr. R. Gaum, Geigy Company, Basle. Dr. J. M. Press, World Health Organization, Geneva. Mr. R. Regamy, World Health Organization, Geneva.
<i>Tanganyika :</i>	Mr. Brian Anderson, Department of Agriculture. Dr. A. C. Evans, Department of Agriculture. Mr. D. Yeo, Arusha.
<i>Thailand :</i>	Mr. K. Jalivacharana, Bangkok.
<i>Trinidad :</i>	Mr. G. K. Malipant, I.C.T.A. Mr. J. Spector, I.C.T.A.
<i>Turkey :</i>	Mrs. C. Otaci, Ankara.
<i>Uganda :</i>	Mrs. L. Mukwaya, Mengo.
<i>U.S.A. :</i>	Professor K. P. Buchholtz, University of Wisconsin. Dr. and Mrs. W. L. Butler, Beltsville, Maryland. Dr. J. E. Carnahan, Wilmington, Delaware. Dr. E. L. Clark, Stamford, Connecticut. Professor M. B. Hoffman, Cornell University, Ithaca, New York. Mr. and Mrs. S. Martinelli, Watsonville, California. Dr. V. H. Olney, Geigy Company, California. Dr. J. van Overbeek, Modesto, California. Dr. Halina J. Presley, University of Illinois. Dr. R. E. Scantlebury, Bethesda, Indiana. Mr. W. Shrimpton, Range Management Associated, San Francisco. Professor F. C. Steward, Cornell University, Ithaca, New York. Dr. G. F. Warren, Purdue University, Indiana. Professor S. H. Wittwer, Michigan. Dr. Gordon Yates, La Crescent, Minnesota.
<i>Yugoslavia :</i>	Mrs. R. Ivkovic, Belgrade.
	Mr. D. Trajanovski, Skopje.
<i>Zanzibar :</i>	Dr. B. E. J. Wheeler, Department of Agriculture.

Shows and Demonstrations.

Exhibits were staged during the year as follows :—

Royal Horticultural Society, Chelsea, May 25th–27th : The use of fluorescent tracers to demonstrate the distribution and retention of spray deposits—arranged by Mr. Mapother in conjunction with Mr. L. N. Staniland of the N.A.A.S.

Wisbech Fruit Show and Marketing Exhibition, 10th/11th November : Apple juice : an outlet for surplus fruit—Dr. Pollard and Miss Kieser ; Some gooseberry and strawberry varieties preserved by canning, deep-freezing, etc.—Miss Leach.

Visiting Scientists.

Professor R. H. Hageman, Associate Professor of Agronomy, College of Agriculture, University of Illinois, U.S.A., is spending a year's sabbatical leave from 1st September, working in the Plant Physiology Section.

Mr. J. F. Bowen, Summerland Research Station, British Columbia, Canada, is spending two years from 3rd October in the Cider and Fruit Juices Section studying the distribution of yeast species and their biochemical activities.

Dr. Milan Milic, Assistant Professor in Agriculture, University of Belgrade, spent two months from 2nd August studying pot culture techniques.

Students and Voluntary Workers.

Mr. M. M. R. Khan Afridi left Long Ashton on 28th September to return to Aligarh Moslem University, India, after studying for a postgraduate degree in the Plant Physiology Section under the direction of Dr. Hewitt since March, 1957. Mr. Afridi was awarded the degree of Ph.D. for a thesis entitled "Some aspects of induced formation of nitrate reductase in plants".

Mr. C. A. Fewson and Mr. G. C. Walker, holders of two-year scholarships from the Ministry of Agriculture, Fisheries and Food, completed their studies under the direction of Dr. Nicholas in October, and were awarded degrees of Ph.D. for the theses : C. A. Fewson : "Some biochemical aspects of bacterial denitrification." G. C. Walker : "Enzymatic studies on the utilization of inorganic nitrogen compounds by *Neurospora crassa* and *Pseudomonas aeruginosa*."

Mr. L. A. Wilson of the University College of the West Indies, Jamaica, commenced a study period at Long Ashton on 1st March under the direction of Dr. Hewitt in the Plant Physiology Section, as a holder of a British Council Scholarship.

Mr. E. J. A. Asomanning of the West African Cocoa Research Institute, Ghana, spent the period 1st February—15th September undertaking training in plant nutrition culture techniques in the Plant Physiology Section.

Mr. H. D. S. Albuquerque and Mr. G. Mohiyuddin completed their year's training in horticultural science and returned to India on 13th October.

By arrangement with Dr. Bond, Mr. O. S. Peries, a post-graduate worker from the Horticultural Science Laboratories, continued his studies on strawberry mildew in the Plant Pathology Section.

The Memorial Studentship in Cidermaking was awarded to Mr. C. J. P. Barnes, a nominee of Herefordshire County Council, who began a ten-month period of study on 1st November.

Social Activities.

The Refectory has been used for a year, to the great benefit of the social activities of the Station. The Staff Christmas Party was held in the building for the first time on Tuesday, 20th December, when members of the Bristol Savages, supported by a few members of staff, provided an enjoyable entertainment.

The Recreational Club suffered a severe blow in the death of its Founder-Chairman, Dr. Bennett. The programme of activities built up during Dr. Bennett's period of office has been continued. Visits to Wellesbourne, the Shakespeare Memorial Theatre at Stratford-on-Avon, Messrs. Showerings at Shepton Mallet, the Police Driving School at Devizes, Messrs. Harveys' vaults and to local theatres, have been well patronized, while some of the more intrepid members visited Steepholme and others explored some of the Mendip Caves. The new Refectory has provided facilities for dances, a garden party, a film show, a demonstration of stereophonic records by Messrs. E.M.I., a firework display on 5th November for the children of staff, and a competition and exhibition organized by the Photographic Section. Treasure Hunts by car have again proved popular, as has a fortnightly Bridge evening. A Magazine Section has just been formed.

The Tennis Club did not have a successful season, because of difficulty in raising sufficient regular players; the committee was therefore obliged to withdraw the ladies' team from the League, and the men's team were relegated to Group 7. The Club's representatives, Mr. Stott and Miss Conibear, both reached the second round of the interclub singles. Eighteen friendly matches were played, of which five were won and three drawn. There was not as much lunch-time tennis played as in previous years, for which the wet weather was largely responsible.

The Table Tennis Club was very popular and membership rose to over forty. In the autumn a team was entered for the first time in the Bristol and District Table Tennis League. The match results were very promising and promotion to a higher Division may be possible next season.

During the 1960 season the Cricket Club missed the services of several veteran players who had left the Station, and rain interfered with many fixtures. Two newcomers, V. Jordan and L. Wilson, are to be congratulated on topping the batting and bowling averages, respectively.

The Rifle Club continued to use the University O.T.C. range for indoor small-bore shooting, and also had permission to use the Backwell Rifle Club range during the winter. During the summer, pistol and full-bore rifle shooting were also enjoyed at the Station range and at Pilning ranges, respectively. The N.R.A. Donegal Badge for 1960 was awarded to Dr. R. A. Webb, and the "News of the World" and "Daily Telegraph" Certificates were won by Dr. Woodcock.

Acknowledgments.

The Station is indebted to the members of staff who have served on staff committees, particularly their chairmen: the late Dr. Bennett and Dr. Webb (Recreational Club), Dr. Burroughs (Library), Miss Kieser (Welfare), Dr. Martin (Laboratory Services) and Dr. Woodcock (Refectory).

Dr. Webb undertook chairmanship of the Recreational Club on the death of Dr. Bennett in May and most successfully organized the season's programme of social activities. The first year of the new Refectory gave rise to a number of problems and Dr. Woodcock was prevailed upon to continue his chairmanship of the Refectory Committee for another year.

Miss P. Batcheller and Mrs. J. Swatridge carried out the task of getting the new kitchen and refectory into smooth running order. The Station is greatly indebted to them and to Dr. Woodcock for their sustained and competent help.

It is particularly pleasing to see the Library refurnished and the staff are indebted to Mr. Colegate, the Librarian, and his assistant, Miss D. Pearce, for the excellent rearrangement of all the books and journals.

Thanks are due to the members of staff who so willingly helped with the extra work that arose in connection with the Station's building programme. In particular, thanks are due to Mr. S. E. Cole, Mr. H. J. Jones, Mr. A. L. Leggett, Dr. H. G. Morgan, Mr. J. H. Russell and Miss M. H. Simpson.

Mr. M. Greenwood again edited the Annual Report, and members of staff are grateful for his most able and kindly help with their manuscripts.

SUMMARY OF RESEARCH, 1960.

Pomology and Plant Breeding.

Plant Breeding.

A new black currant variety which combines earliness with good travelling qualities and other desirable characters has been named Tor Cross ; it is being propagated for limited distribution in 1962. Two families of seedlings have been produced by crossing the Scandinavian black currant variety Brödrtorp with Baldwin and Wellington XXX, in an attempt to combine the high yield and earliness of Brödrtorp with the upright habit of the two other varieties. In a further series of interspecific crosses with *Ribes nigrum* as the female parent, hybrid seed was produced in 34 out of the 45 crosses attempted. Sixteen species of *Ribes* have been successfully grafted on reverted black currant stocks with the combined objects of testing them for tolerance to reversion and of searching for a sensitive indicator for reversion virus.

More of the 1947 series of cider apple seedlings fruited for the first time, and of 39 evaluated 9 were retained for further observation. One of this family of seedlings that showed promise in a small-scale cider-making trial last year is being propagated for further trials. Amongst other seedling trees which cropped for the first time this year, one early dessert apple and one pear are being retained for further observation. A series of apple progenies was raised for studies of the inheritance of tolerance to 2,4-dichlorophenoxyacetic acid (2,4-D) and for further testing the apparent ability of the apomictic *Malus* rootstocks to shorten the juvenile phase. In three of these crosses, using Laxton's Fortune as female parent, there was some suggestion of a xenia effect for fruit size and colour.

In an experiment using the spur-unit method of pollination, variations in the rate and pattern of fruit drop were observed in Cox's Orange Pippin pollinated by four different varieties. Unfortunately, the low overall set on Cox in 1960 precluded any statistical comparison of the effects of different pollinators. Further pollination experiments with *Malus sikkimensis*, *M. toringoides* and *M. theifera* confirmed that all these species are facultative apomicts. Fruit-set and seed number were increased when the flowers were hand pollinated with apple pollen (Lord Lambourne), but foreign pollen (*Crataegus monogyna*) was ineffective.

Growth Substances.

In collaboration with the Physical Chemistry section, studies of the metabolism of ^{14}C carboxyl-labelled 2,4-dichlorophenoxyacetic acid (2,4-D) were extended to cover a wide range of crop plants and weeds. It was found that, in general, tolerance to this herbicide is associated with the ability of the plant to metabolise the 2,4-D rapidly to other compounds, though at least one exception to this general rule was found, viz. *Urtica dioica*, which, although able to metabolise 2,4-D rapidly, is classified as a sensitive species.

In connection with problems arising in the field application of 1-naphthaleneacetic acid (NAA) for the control of fruit drop and the thinning of apples, studies were made of the uptake of this compound by the apple leaf and of its subsequent metabolism. The appreciable volatility of NAA necessitated considerable modification of the techniques developed for 2,4-D.

At 25°C, approximately 30 per cent of the NAA on the leaf surface is absorbed during the first two hours after spraying. Absorption continues even after the leaf is dry, though at a slower rate dependent on temperature and on the relative humidity of the air. Once inside the leaf, over 90 per cent of the NAA is changed in a few hours to another compound, and this subsequently changes more slowly into a third compound. Which of these various metabolic products is responsible for the growth-regulating effects of NAA is not at present known.

In a field trial with maleic hydrazide for the inhibition of runner development in maiden strawberries, promising results were obtained with the varieties Talisman and Cambridge Vigour. Good runner control was also obtained with Cambridge Favourite, but the plants suffered considerable damage. With Redgauntlet both runner control and damage were slight.

The effect of three persistent residual herbicides on the growth in pure culture of several species of nitrogen-fixing and nitrifying bacteria was investigated with the collaboration of the Chemical Microbiology section. Pure simazine (2-chloro-4,6-bis(ethylamino)-1,3,5-triazine), atrazine (2-chloro-4-isopropylamino-6-ethylamino-s-triazine) and diuron (N-(3,4-dichlorophenyl)-N'-dimethyl urea) in amounts in excess of those normally used in the field, were found to be without effect on the growth of these organisms. Formulated preparations of these herbicides, however, inhibited growth, presumably because of the presence of dispersing agents.

The tolerance of various types of fruit plants to some of the newer residual herbicides was investigated by growing the plants in sand culture and watering them with solutions of the herbicides. Monuron (N-(*p*-chlorophenyl)-N'-dimethyl urea) and diuron under these conditions damaged all types of fruit plants with the exception of certain varieties of apple (e.g. Bramley). Simazine severely damaged black and red currants and caused slight damage on pears and raspberries but the apple and plum varieties tested appeared to possess a high degree of tolerance to simazine.

In field trials with simazine at 6 lb./acre for weed control in black currants, no damage was caused to the bushes and the yield was equal to that from handweeded plots. Simazine at 3 and 6 lb./acre applied as a residual herbicide to plots containing a wide range of apple and pear rootstocks and scion varieties caused no damage and gave practically complete weed control throughout the season. Weed control was not apparently affected by rotovation. In the same experiment diuron at 6 lb./acre caused slight leaf scorch in Quince A, seedling apple and Malling IX rootstocks.

In order to study the relative persistence and penetration of residual herbicides in Long Ashton soils, a large replicated trial was laid down in which seven herbicides, each at three rates of application, were applied to clean soil at the beginning of July. Simazine and atrazine proved the most persistent, followed by diuron and monuron. The effects of chlorpropham (isopropyl-*m*-chlorocarbanilate), amiben (3-amino-2,5-dichlorobenzoic acid) and 2,4-DES (sodium 2-(2,4-dichlorophenoxy)ethyl sulphate) were relatively short lived under the heavy rainfall during the latter half of 1960.

In the search for a chemical method of controlling grass in the unmown area around the bases of the trees in orchards, four herbicides, each at three different rates of application, were applied in April to a rather weedy rye grass/clover sward; some treatments were repeated later in the season. All treatments caused some reduction in the growth of the sward, the most promising

being paraquat (1,1'-dimethyl-4,4'-bipyridylium-di(methyl sulphate)): at only 1 lb./acre this compound reduced by 50 per cent the fresh weight of herbage cut in October and caused a marked increase in the clover content of the sward. 6 lb./acre of dalapon (sodium 2,2-dichloropropionate) also reduced the total herbage by 50 per cent, but these plots were heavily invaded by perennial weeds. 3-amino-1,2,4-triazole had a temporary inhibiting effect whilst maleic hydrazide was the least effective of the compounds tested.

Physiology of Fruit Plants.

Investigation of the cause of abnormal bud-break on apple trees growing in controlled environment chambers (Report for 1959, p. 23) has shown that humidity and watering are not the major factors involved. An experiment is now in progress to determine the effect of temperature regime on the bud burst and flowering of three varieties of apple.

Experience in fruit thinning, both by hand and chemical means, has shown that the response, as measured by increase in fruit size, varies from year to year. In an attempt to determine the factors influencing fruit size and response to thinning, four varieties of dessert apple are being studied. The cropping pattern of thinned and unthinned branches is being surveyed with the object of relating it to previous cropping history and weather conditions, and of analysing these factors in terms of their effect on time of flower initiation, cortical cell number and cell size, and seed content of fruits.

Investigations of pollen tube growth and ovule development were made in several apple and pear orchards in which fruit-set was known to be unsatisfactory. In several orchards the low initial set was shown to be due to inadequate cross-pollination; in others female sterility appeared to be the chief cause. Thus, in one orchard of Broad-nosed Jersey (Ditchet type) less than one flower in 20 contained receptive ovules at full bloom and some material of Cox's Orange Pippin and the pear variety Conference was only slightly better.

Cox's Orange Pippin apples from the cover crop experiment at Long Ashton (Plot 18) were used in a study of fruit quality and storage life in relation to cover crop and manurial treatment. Fruits with the highest nitrogen concentration were the largest; they also had the lowest seed number, the highest cell number, the greatest loss from storage disorders and were preferred above all others by a tasting panel. Contrary to expectations the greatest loss in weight during storage was shown by the fruits with a high potassium content. In Cox, Laxton's Superb and Bramley it was established that there was no significant after-ripening of the seeds during the time the fruit was in cold storage. Natural growth-promoting substances were detected in the flesh of ripening apples and further studies are in progress to determine whether these play any part in controlling storage behaviour.

Variety Trials and Cultural Experiments.

In the cropping trial with the virus-tested sub-clone of the loganberry LY 59 the gross yield per acre in 1960 was 6.2 tons per acre compared with 4.7 tons in 1959, but owing to damage by wind and rain the marketed crop was approximately the same as in 1959 (4.3 tons/acre). At the present spacing of 6 ft. in the row, only about one-quarter of the cane produced can be accommodated on the wires after pruning and tying-in; some plots have therefore been thinned to 12 ft. for further study.

Plants of two further virus-tested loganberry clones (L.463/3 and L.460/2) have been received from East Malling and will be propagated at Long Ashton in 1961; they will later be planted in the field to measure their performance against clone LY 59.

A detailed botanical analysis of the swards in the Long Ashton cover crop experiment (Plot 18) was carried out in 1960; this was the third analysis since the cover crops were sown eight years ago. The most striking changes during this period have been a progressive decrease in the density of white clover on the clover plots, and a marked build-up of white clover and *Agrostis stolonifera* on the plots sown with perennial rye grass. On the "tumble-down" plots *Poa annua* was dominant for the first six years but this species has now been largely replaced by *Agrostis*, white clover and rye grass. The most stable sward is that sown to timothy (*Phleum pratense*) which, apart from some invasion by clover and the disappearance of annual weeds, shows much the same botanical composition as it did in 1954.

Rootstocks and Propagation.

The juvenile phase of apple seedlings, which normally lasts from 7 to 14 years, has been shortened considerably by budding on seedling stocks of the apomictic *Malus sikkimensis*. In a field trial with three different families of seedlings, 53 per cent flowered in 1960—the fourth year after budding. None of the unworked seedlings planted alongside have yet flowered. This technique seems likely to prove of great value in top-fruit breeding, where the length of the juvenile phase has always been a limitation.

Observations have continued on a small pilot trial of dessert apples on apomictic seedling rootstocks, and two of these stocks, *Malus sikkimensis* and *M. toringoides*, have been included in a larger trial in which comparisons will be made with the clonal stocks MM.104, MM.111 and M.II. The scion varieties in this new trial are Cox, Worcester and Jonathan.

In a search for healthy propagating material of cider and dessert apples, trees in many parts of the country have been indexed for latent viruses, using *Malus platycarpa*; they were almost invariably found to be infected. Scions from the original seedling tree of Bramley's Seedling, now over 100 years old, proved, however, to be free from all known viruses, as did the new clonal rootstocks MM.104, MM.111 and M.26. Work was continued on the heat treatment of apple rootstock and scion varieties with the object of eliminating latent viruses. 20 days at 98°F failed to inactivate the *platycarpa* group of latents, although the virus concentration was reduced by this treatment. Heat treatment is now being carried out for longer periods and followed by tip propagation.

M. platycarpa line pattern virus has also been found in the pear varieties Bristol Cross, Blakeney Red and Gin, where it is associated with large ring spots on the leaves. The same virus occurs in some plums, but is not identical with plum line-pattern virus. Of 19 varieties of pear virus-tested on Lord Lambourne, six were found to be carrying apple rubbery wood virus. Further work on indicator plants for pear viruses has suggested that selected pear seedlings may be of value. Eight such virus-sensitive seedlings have been vegetatively propagated for further tests.

The sensitivity of a wide range of *Malus* species to the *platycarpa* group of latent viruses has been investigated. These viruses proved lethal to *M.*

atrosanguinea, *M. floribunda*, *M. fusca*, *M. Prattii*, *M. Sieboldii*, *M. glaucescens*, *M. denticulata*, *M. prunifolia* var. *Rinkii*, *M. florentina*, *M. Sargentii*, and *M. Lemoinei*; a further 20 species were severely stunted. This explains the failure of nurserymen to raise trees of these ornamental species on the Malling clonal rootstocks, which are now known to be carriers of this group of viruses. It may also explain the slow-decline of commercial apples worked on *M. prunifolia* stocks, which has become a serious problem in Japan.

Scions and bud-stocks of the Long Ashton clone of *M. platycarpa* have been sent on request to 20 research institutes in various parts of the world.

An attempt was made to transmit bitter pit by bud inoculation to families of seedling apples produced by crossing varieties known to be susceptible to this disease. Bitter pit has shown up both on the inoculated trees and on the uninoculated controls, which makes it highly unlikely that this disease is of a virus nature.

A long-term propagation experiment on black currant has shown that the treatment of bushes in the nursery can affect their subsequent cropping in the plantation. Bushes transplanted in the nursery have given a consistently lower crop over a three-year period than non-transplanted bushes.

Cider and Perry Varieties.

During 1960 only small supplies of grafts and bud-sticks of perry pear varieties were sent out to growers. The perry pear propagation scheme will now be discontinued as nurseries are now able to produce their own material. Future work at Long Ashton will be concentrated on providing virus-tested propagating material of cider and perry varieties.

Pomological descriptions of cider apple varieties are being prepared for publication and a dichotomous key for the identification of the principal varieties of perry pear has been drawn up.

See also Nos. 1, 20, 33, 34 and 35 in *Publications, 1960*, pp. 47-50.

Plant Nutrition : Fruit.

Field Experiments.

The long-term nutritional experiments at Long Ashton (apple and pear), and the N.A.A.S. Experimental Horticulture Stations at Efford (apple, pear, black currant) and Luddington (plum and black currant) have been continued. In the cover-crop-nutrition apple experiment at Efford (started 1955) the only significant effect of fertilizers on leaf composition yet obtained has been due to the omission of potassium. The percentages of potassium in dry matter were : $+K=1.38$; $-K=1.27$, for Cox's O.P. and $+K=1.31$; $-K=1.22$ for Worcester leaves sampled in August 1958. The omission of potassium fertilizer from Worcester trees had a significant (5% level) depressing effect on the yield of marketable fruit in 1959. No leaf analysis data are available for 1959, but it would appear from the previous year's results that a leaf potassium value of 1.2%, in samples taken from the middle-third region of extension shoots in August, is below the sufficiency level for apple. Leaf nitrogen concentrations in 1958 were high, and leaf phosphate values were about normal. Visual observations in 1960 showed that the cover crop, sown in 1959, reduced the leaf nitrogen status of both varieties compared with that of trees under arable culture.

Crop yields and leaf analyses are now available (p. 69) for the second phase of the NPK experiment on strawberry (var. Favourite) under cloches at Rosewarne Experimental Horticulture Station. Fertilizers had no significant effect on yield of fruit, thus confirming the previous findings with the variety Royal Sovereign. Chemical analysis showed that the concentrations of total N, P and K in leaves taken from control plants (no fertilizer) at the fruiting stage were above the sufficiency levels. These results are in agreement with previous observations that virus-tested strawberries have a low fertilizer requirement, particularly if cropped for one season only.

The first season's results from four new experiments designed, in collaboration with Mr. F. A. Roach of the N.A.A.S., to study the effect of time of application of nitrogenous fertilizers on strawberry, showed no significant effect of treatments on crop yield. Chemical analysis of leaves, taken at the fruiting stage, showed that the nitrogen status in plants from control plots (no nitrogenous fertilizer) was above the sufficiency value. It would appear, therefore, that when the level of nitrogen in plants is adequate, as determined by leaf analysis, the time of application of fertilizer-nitrogen has no significant effect on the subsequent yield of fruit.

Pot Experiments.

Further experiments have been carried out with strawberry, var. Royal Sovereign, to determine the sufficiency level of leaf magnesium at the fruiting stage. Previous experiments (1959) had shown that the concentration in dry matter could be varied between 0.14 and 0.39% Mg without significantly affecting the yield of fruit. Current season's work showed that the yield of fruit was reduced significantly when the leaf-Mg at the fruiting stage fell below 0.1% in dry matter; magnesium deficiency symptoms appeared in the leaves when the concentration reached 0.06% Mg in dry matter. It would appear from these results that strawberry has a lower Mg-requirement than apple.

Sand culture experiments were started with the trace elements copper, iron, manganese and zinc to determine the range over which the supply of these elements affects growth and yield of fruit, and at what leaf concentration visible symptoms of deficiency or excess appear. It is hoped to correlate chemical leaf composition, at specific stages, with growth and crop yield in the same manner as for the major nutrients. Sub-irrigation methods of sand culture with strawberry have been investigated; difficulties have arisen which will need to be overcome before this semi-automatic method can be adopted.

Two comprehensive factorial sand culture experiments ($N \times P$ and $K \times Mg$) were started with raspberry, var. Lloyd George, to study the relationship between chemical leaf composition, growth and crop yield.

Experiments to determine the effect of time of application of nitrogen on maiden apple trees (Lord Lambourne) in sand culture were continued. Nitrogen applied during the early summer of 1959 induced only a small increase in growth but a considerable increase in the proportion of flower to vegetative buds in the spring of 1960, compared with controls lacking nitrogen. Late summer applications induced more growth, but the extra buds produced were predominantly vegetative. Nitrogen added at bud-break in 1960 did not alter the proportion of flower buds produced, compared with the controls.

Further experiments, employing more and shorter periods of application of nitrogen, were carried out during 1960, and the effects will be studied in 1961.

Studies of the uptake and metabolism of iron chelates have been continued in collaboration with the Physical Chemistry section. It has been shown that iron and the chelating agent are not always absorbed in equivalent amounts by plants. Iron-sufficient plants absorbed iron and the chelating agent (ethylenediamine bis-(*o*-hydroxyphenylacetic) acid) in approximately equivalent amounts, but iron-deficient plants initially absorbed much larger amounts of iron than chelating agent. This independent absorption did not, however, take place in iron-deficient plants in the presence of 10 m.e./litre of bicarbonate.

When plants were grown in nutrient cultures containing ferric ethylenediaminetetra-acetate (Fe-EDTA) labelled with radioactive iron (^{59}Fe) and radioactive carbon (^{14}C) there was a progressive total loss of ^{14}C with time, whereas a complete recovery of ^{59}Fe was made, from solution and plants. This is the first experimental evidence for the decomposition of chelates by plants. Further studies on the mechanism of decomposition are being made.

Preliminary experiments were made to investigate the factors concerned in the uptake of phosphorus by P-deficient strawberry plants from soil low in available P. In collaboration with the Cider section attempts were made to sterilize strawberry roots, without causing damage, for studies of root excretions.

Laboratory Investigations.

Work on leaf analysis as a guide to the nutritional status of crops was continued, using leaf samples from long-term factorial manurial experiments and pot cultures. It has been shown that there is no loss of nitrogen on storage for one year of dried samples of apple, black currant and strawberry leaves dried initially at 65°C and 105°C ; there is some evidence that losses occur after prolonged storage.

In connection with the work on iron chelates, chromatograms of extracts from plants grown with ^{14}C -labelled Fe-EDTA have shown the presence of radioactive substances other than Fe-EDTA or EDTA. These have not yet been identified. A study has been made of the photo-decomposition of iron chelates *in vitro*. It is suggested that the breakdown of the ligand is caused by an attack by free OH radicals formed during photo-reduction of the iron.

A method is being developed for the quantitative determination of copper, manganese, iron and zinc in plant materials, using paper chromatography. The ashed material, after conversion to chlorides, is solvent extracted, the four elements are separated in one step and assayed colorimetrically after elution from the paper. For quantities less than $3\text{ }\mu\text{g.}$ the element is assayed directly on the paper by a reflectance method; between $0.15\text{ }\mu\text{g.}$ and $3\text{ }\mu\text{g.}$ there is a quantitative relationship between the percentage reflection of light from the coloured spot and the amount of the element present.

Further work on the Titan Yellow method for the determination of magnesium has resulted in a procedure more rapid than the one previously reported. A new chelating agent, ethylene glycol bis-(β -aminoethylether)-NN' tetraacetic acid (EGTA), has been used to complex calcium selectively without preventing the precipitation of magnesium hydroxide. Under these conditions,

no turbidity develops in solutions high in phosphate, which need therefore not be removed by ion exchange prior to the determination.

See also Nos. 4, 5, 6 and 7 in *Publications, 1960*, pp. 47-50.

Organic Chemistry.

In further investigation of the roles played by chelation and lipid solubility in the fungistatic activity of 8-hydroxyquinoline ("oxine"), the eight 6-*n*-alkyl oxines prepared in 1959 (19) have now been tested against the mycelium of *Aspergillus niger*. They showed maximum activity at a chain length of 6-7 carbon atoms, except in the presence of Cu^{++} , when oxine and 6-methyl oxine were found to be most active. It is interesting to note that, when unchelated, 6-*n*-hexyl oxine was more active than 5-*n*-hexyl oxine, its ED_{50} being approximately one-half. Cases of anomalous growth inhibition in the presence of Cu^{++} have been shown to be due to the production of oxalic acid by *A. niger*, resulting in the liberation of the free oxines from the metal complexes.

Work on the fungitoxicity of 2,4-dinitro-6-(1'-methylheptyl)phenyl crotonate (dinocap, the active principle of Karathane) has been continued. The *n*-pentyl, *n*-hexyl and *n*-heptyl members of the series of 2,4-dinitro-6-*n*-alkylphenyl crotonates are liquid and at first failed to give satisfactory microanalyses. They have now been freed from traces of the parent phenols, probably formed pyrolytically on distillation prior to analysis, by percolation through a column of activated alumina. A similar method has been used for the purification of dinocap, the isomeric 2,4-dinitro-6-(1'-*n*-propylpentyl)-phenyl crotonate, and the corresponding butyrates. Encouraging results in preliminary anti-fungal assays have led to the synthesis of 2-alkyl-4-nitro-1-naphthol derivatives related to dinocap; the preparation of a number of esters of 2,4-dinitro-1-naphthol is in progress. Other compounds that have been prepared for testing include the butyrate and crotonate of 4-nitro-1-naphthol and the cinnamates of 2-nitro- and 2,4-dinitrophenol.

A series of *n*-alkylguanidines has been prepared for an investigation of the fungitoxicity of *n*-dodecylguanidine acetate (dodine (Cyprex/Melprex)) and of the effect of alternative anions.

In the metabolism of 2,4-dichlorophenoxyacetic acid by *A. niger*, the major product has been shown to be a hydroxy-2,4-dichlorophenoxyacetic acid not identical with the expected 6-hydroxy acid. Work on the orientation of this compound and on the identification of two other acidic metabolites is in progress. The hydroxylation of 4-chlorophenoxyacetic acid by *A. niger* has been shown to give not only the expected 4-chloro-2-hydroxyphenoxyacetic acid but, rather surprisingly, the *m*-substituted metabolite, i.e. the previously unknown 4-chloro-3-hydroxyphenoxyacetic acid, which has also been synthesised for the first time for unequivocal identification. The metabolism of 2-chlorophenoxyacetic acid by *A. niger* has produced four major products, as indicated chromatographically. Three of these have been identified as the hitherto unknown chlorohydroxyphenoxyacetic acids, of which the 2-chloro-5-hydroxyphenoxyacetic acid is produced in greatest amount. This is another example of unusual *m*-hydroxylation. 2-Chloro-3-hydroxyphenoxyacetic acid and the corresponding 6-hydroxy compound have also been identified chromatographically. The fourth acid has an R_F value different from that of the known 2-chloro-4-hydroxyphenoxyacetic acid, and has not yet been identified.

See also Nos. 19 and 64 in *Publications, 1960*, pp. 47-50.

Physical Chemistry.

The two major projects undertaken with radioactive tracers have again been investigations of the metabolism of growth-substances, made in collaboration with the Pomology section, and of the metabolism of iron chelates made with the Plant Nutrition section. Using compounds labelled with ^{14}C , it has been shown that the resistance of a wide variety of plants to 2,4-D is associated with their ability to metabolise the compound. Factors influencing the absorption of NAA by apple leaves have also been investigated; absorption has been shown to be followed by rapid metabolism. (See also p. 22). The chelate and iron fractions of added iron chelates labelled with ^{14}C and ^{44}Fe have been found to be differentially absorbed by plants that are iron-deficient at the time of treatment. Such unequal absorption did not occur in normal plants, or in iron-deficient plants grown in a bicarbonate solution. The first experimental evidence for the decomposition within the plant of the chelate fraction of an applied iron chelate has also been obtained. (See also p. 28).

Assistance with the preparation and assay of radioactive solutions (^{14}C , ^{32}P , ^{58}Co , ^{59}Fe , ^{64}Cu , ^{99}Mo) has again been given to the Chemical Microbiology, Analytical and General Chemistry, Plant Physiology and Plant Pathology sections, and also to the National Vegetable Research Station (^{65}Zn).

Further modifications in the oscillating jet method for the determination of dynamic surface tensions include a procedure that enables results to be obtained that are independent of the orifice used.

The dynamic tensions of solutions of commercially pure sodium di-(2-ethyl hexyl) sulphosuccinate (DEHS) and sodium dinonyl sulphosuccinate (DNS) have been determined at concentrations of 0.025% and 0.05% w/v in distilled water. At 0.025% the two salts age at about the same rate for the first 1/20th sec. but at 0.05% DNS ages more rapidly. Since in practice wetters are used in waters of different degrees of hardness, experiments were carried out using a typical soft water (7 p.p.m. Ca) and Bristol tap water (73 p.p.m. Ca). Although the static tensions of solutions in these waters were very similar, striking differences were found in the initial rates of fall of surface tension. In soft water the initial rate of fall of 0.05% DEHS was greater than in distilled water, but in Bristol tap water the initial rate was considerably less. With DNS the effect was even more marked, and minute amounts of calcium in the water retarded ageing. In Bristol tap water the tension of a 0.05% solution of DNS was still greater than 65 dynes/cm. after 1/20 sec. whilst in distilled water the tension had fallen to 40 dynes/cm. in 1/40 sec.; this behaviour is associated with the precipitation of the sulphosuccinate as its calcium salt. The results emphasize the inadequacy of static tensions for appraising the suitability of a surface-active compound for any process where rapid expansion or renewal of surface occurs.

See also Nos. 34 and 35 in *Publications*, 1960, pp. 47-50.

Analytical and General Chemistry.

As previously observed with *Alternaria tenuis* (Report for 1959, p. 29), the electro-negativity values of some twenty-four cations were found to govern their toxicities to *Botrytis fabae*. Experiments were continued on the uptake of ^{64}Cu by fungal cells; after disintegration the ^{64}Cu was found to be non-specifically associated with the particulate cellular fractions, the amount of ^{64}Cu combined with the cell fractions varying greatly with the spore species.

Further studies of the uptake of captan (N-trichloromethylthio-*cyclohex-4-ene-1,2-dicarboxyimide*) showed large variations in the uptake, on a spore weight basis, by spores of twelve different fungal species. The uptake by *Neurospora crassa* was reduced in the absence of oxygen and increased in salt solutions. Pre-treatment of *N. crassa* spores with thiol-reacting compounds, such as iodoacetic acid, greatly reduced captan uptake without lessening the toxic effect, which suggests that most of the captan normally absorbed is inactivated by -SH groups within the cell. The tenacity and fungitoxicity of a number of particle-size fractions of captan were found to increase with decreasing particle size. The tenacity of the crystals was dependent on particle shape as well as size.

The method for the determination of micro-quantities of dieldrin (Report for 1959, p. 29) was shown to be applicable to endrin (50). The micro-determination of sulphur on plant material (Report for 1956, p. 122) was re-examined and determinations of lime sulphur deposits on black currant shoots were carried out for the Entomology section in connection with the control of the Big Bud mite. A rapid method was devised for the determination of dinocap deposits on strawberry fruits and apple foliage, soils were assayed for DDT for the National Vegetable Research Station and work was continued on the standardization of alumina used for the removal of interference by plant constituents in analytical methods for spray residues.

Investigations were continued on the contamination of crops by mercury compounds used for disease control. Conjoint work with the Mercury Panel of the Ministry of Agriculture's Scientific Subcommittee on toxic chemicals resulted in an agreed method of analysis for mercury in apples and tomatoes. The conditions necessary for the complete recovery of mercury in the analysis of soils were determined. The presence of mercury in the seeds, in pod, of broad bean plants treated with phenylmercuric acetate at flowering time was confirmed, and indications were again obtained that mercury was translocated into apple fruits from the foliage of sprayed trees. Special attention was given to the uptake by plants of mercury from soils treated with calomel, mercuric chloride or mercuric oxide for club-root control; collaborative work with the Rosewarne Experimental Horticulture Station and the West of Scotland Agricultural College showed some uptake of mercury by the roots of lettuce, French bean and carrot. Tree bark samples were examined for mercury for the Plant Pathology section in connection with the control of *Gloeosporium perennans*, and lime leaves and fruits were analysed for the Department of Agriculture, Zanzibar, in work on the control of *G. limetticola*. Some of the investigations on mercury are reported more fully on pp. 85 and 111.

Further work was carried out on the cuticles of plants in relation to the deposition and penetration of spray deposits and susceptibility to disease. Improved methods were used for the recovery and fractionation of the surface waxes from leaves and fruits. Changes were followed in the waxes and cutin in the upper and lower surfaces of leaves of different ages and of leaves showing differing degrees of infection by fungi. The cuticles of apple fruits were examined in relation to damage by mercury and storage behaviour and those of lime leaves and coffee berries in relation to disease infection. Detailed comparisons were made of the cuticles of leaves of varieties of apple, strawberry and rose, resistant and susceptible to powdery mildews. The degradation of cutin in the leaf cuticle by mildews and the apple scab fungus was shown

quantitatively. Some fractions of surface leaf waxes stimulated the germination of mildew spores; by contrast, the cutin acids obtained from the cutin showed fungicidal effects. The cuticle work is described more fully on p. 102.

See also Nos. 2, 37, 38, 39, 40, 41, 47, 50, 51, 52, 53 and 54 in *Publications, 1960*, pp. 47-50.

Plant Biochemistry.

Studies of the photochemical reactions of isolated chloroplasts (Report for 1959, p. 44) have provided the basis for an investigation of the mode of action of those of the newer herbicides that have been shown to function through their effect on photosynthesis.

In particular, the defoliant diquat (1,1'-ethylene-2,2'-dipyridylum dibromide) is known to be herbicidally effective only in tissues that contain chlorophyll and only when they are exposed to the light. Preliminary experiments with isolated chloroplasts showed that diquat is without effect on the photochemical reduction of a number of artificial hydrogen acceptors including ferricyanide. By contrast, the reduction of triphosphopyridine nucleotide (TPN), catalysed by a specific leaf protein fraction (26), is completely inhibited at low concentrations (10^{-4} M) of the herbicide. This reduction is generally thought to be a key reaction in the light phase of photosynthesis. The inhibition by diquat was found to be competitive, reduction of the herbicide occurring preferentially. Since reduced diquat is rapidly re-oxidised by molecular oxygen, the rate of this reduction by the illuminated chloroplasts could not be followed by direct methods. Re-oxidation results, however, in the production of hydrogen peroxide, which could be detected spectroscopically as a complex with metmyoglobin. Diquat can therefore function as a catalyst of oxygen reduction by illuminated chloroplasts.

By trapping the hydrogen peroxide in the presence of catalase and ethanol, the reduction of oxygen was measured manometrically as an oxygen uptake. The reaction is strongly stimulated by the further presence of a phosphate acceptor system containing adenosine diphosphate, orthophosphate and Mg^{++} , when adenosine triphosphate (ATP) is produced in amounts stoichiometric with the oxygen taken up. Diquat thus appears to catalyse a cyclic photophosphorylation coupled to the reduction of oxygen.

Cyclic photophosphorylation has been suggested as a mechanism whereby a photosynthesizing system is able to produce the amounts of ATP required for the operation of those enzymes involved in the "dark" reduction of carbon dioxide; two naturally occurring substances, vitamin K and riboflavin-5-phosphate (FMN), have been suggested as "natural co-factors". Experiments with FMN and menadione, an analogue of vitamin K, have shown that both are active in catalysing the reduction of oxygen, with the production of hydrogen peroxide, and that this reduction can be coupled to phosphorylation to give phosphate and oxygen uptakes in the ratio of 1 g. atom P to 1 g. atom O. Two redox indicators, methyl and benzyl viologens, substances structurally related to diquat, gave similar results. It was found, moreover, that all these substances, with the exception of FMN, possessed herbicidal properties. Since oxygen does not appear to serve as hydrogen acceptor during normal photosynthesis in green algae, the experiments reported here suggest that cyclic phosphorylation may not be a natural process. This possibility is under examination.

See also Nos. 23, 24, 25 and 26 in *Publications, 1960*, pp. 47-50.

Plant Physiology.

Work on induction of nitrate reductase activity was continued with cauliflower plants grown in sterile cultures to prevent nitrification. Nitrate reductase activity could not be detected in plants grown with glutamic acid and added molybdenum but appeared in detached leaves within two or three hours after infiltration with nitrate. Activity is lost from plants deprived of nitrate for two or three days and is restored on infiltration of nitrate, but not of ammonium compounds, into detached leaves. The effects of temperature and of increasing concentrations of molybdenum or nitrate as inducers have been investigated.

An enzyme system which causes rapid oxidation of hydroxylamine by extracts of plant leaves and roots has been identified as a peroxidase system dependent on manganese and a monophenol co-factor such as *p*-cresol or 2,4-dichlorophenol; resorcinol is also effective. The activities of this system and of hydroxylamine reductase are distinguished by the use of anaerobic conditions. Reduction of nitrite by detached intact leaf tissues is dependent on light.

In collaboration with Professor R. H. Hageman of the University of Illinois, consistently high nitrite reductase activities have been obtained with preparations from vegetable marrow when tested under anaerobic conditions. The electron donors, co-factor requirements and reaction products of these systems are under investigation. The activity of nitrate reductase in leaf extracts depends upon the concentration of cysteine present at the time of extraction, and the optimum level required varies with species and growth conditions.

In collaboration with Dr. G. Bond of the University of Glasgow, molybdenum has been shown to be essential for nitrogen fixation in the root nodules of non-legumes, including *Casuarina Cunninghamiana*, *Alnus glutinosa* and *Myrica gale*. Molybdenum accumulates up to 200 p.p.m. dry weight in root nodules and 20-50 p.p.m. in other tissues. These levels are higher than those usually found in legumes under similar conditions. First experiments to test the effects of cobalt on nitrogen fixation in these species have been inconclusive.

An improved method has been tested for measuring cell numbers in leaf primordia of tomato and tobacco plants. It is now possible to apply haemocytometer counts to all leaves except the first or second primordium. The method for primordial leaves differs from that of Sunderland & Brown in using EDTA 0.1 M in 5% aqueous butanol pH 8.0 in addition to pre-treatment with acetic alcohol and *N* HCl for leaves up to 1 cm. in length. Chromic acid is used for larger leaves up to maturity. A linear relationship between logarithm of cell number and primordial position has been observed up to the seventh or eighth leaf in tomato and up to the eighth or ninth leaf in tobacco grown in water culture. The effects of zinc supply on leaf growth and protein content are under investigation.

A method for the estimation of ribose- and deoxyribose-nucleic acids and of protein in a single small sample of leaf tissue is being developed with special reference to factors affecting degradation of the macro-molecules and their contamination by phosphorus compounds of low molecular weight during separation.

See also Nos. 22, 29 and 30 in *Publications, 1960*, pp. 47-50.

Chemical Microbiology.

Studies of the inorganic nitrogen metabolism of soil bacteria were continued.

Denitrification. Enzymes concerned in the conversion of nitrate to nitrogen gas were characterised in *Pseudomonas aeruginosa*. An active dissimilatory nitrate reductase purified more than 100-fold from this bacterium was shown to require reduced diphosphopyridine nucleotide as an electron donor and to contain flavin adenine dinucleotide, cytochrome c, molybdenum and sulphhydryl groups as functional constituents. The sequence of electron transfer to nitrate or oxygen as alternative terminal acceptors is from reduced diphosphopyridine nucleotide via flavin adenine dinucleotide and cytochrome c to either the Mo/NO_3 or the cytochrome oxidase/ O_2 acceptor.

A nitrite reductase purified 600-fold from the same bacterium was shown to utilize a range of electron donors including reduced flavins, methylene blue and pyocyanine, but not the reduced pyridine nucleotides. The enzyme, which was assayed anaerobically, contains cytochrome c, copper and sulphhydryl groups and requires either phosphate or sulphate for maximal activity. The product of enzyme reduction is nitric oxide.

A nitric oxide reductase, purified 20-fold from *Pseudomonas*, is an iron-containing flavoprotein which utilizes a number of electron donors, other than the reduced pyridine nucleotides. Nitrogen gas is the reduction product. Nitric oxide is readily utilized by extracts of micro-organisms or higher plants grown on nitrate but there is little uptake when the organisms have been given ammonia as the sole nitrogen source. Nitric oxide is also utilized by bacteria, including the rhizobia of root nodules, and by blue green algae, when fixing atmospheric nitrogen.

Nitrification. Cell-free extracts of *Nitrosomonas europaeae* (culture kindly supplied by Dr. Jane Meiklejohn, Rothamsted Experimental Station) were shown to oxidise hydroxylamine to nitrite. The first step appears to be a dehydrogenation reaction that requires a suitable acceptor, e.g. methylene blue, and produces nitric oxide under anaerobic conditions. Methods were developed for the quantitative determination of hyponitrite, nitric oxide and nitrate. Cell-free extracts of *Nitrobacter agili* were shown to oxidise nitrite to nitrate. Cytochrome c and its oxidase are essential for this reaction and inorganic iron was also found stimulatory.

Nitrogen Fixation. Cell-free extracts of *Azotobacter vinelandii* were found (Report for 1959, p. 44) to incorporate substantial amounts of the stable isotope, nitrogen-15. Radioactive nitrogen (^{15}N) is being used in collaboration with the M.R.C. Radiotherapeutics Unit, Hammersmith Hospital, to study the incorporation of nitrogen into these extracts.

See also Nos. 27, 28, 42, 43, 44, 45, 46 and 57 in *Publications, 1960*, pp. 47-50.

Plant Constituents.

The uptake of simple phenolic substances by detached leaves and young shoots of *Malus* and related genera has been studied as part of a more general investigation of the formation and distribution of phenolics within the plant. Simple dihydric phenols are taken up easily, and largely converted to glycosides; but quite small increases in the complexity of the molecule seem to decrease or stop the uptake. Phenolic acids appear to be taken up more easily

from neutral buffered solutions than from plain aqueous solutions ; the extent of uptake and of glycosidation vary greatly, however, with the individual acid.

An examination has been made of the distribution of phenolic substances within growing shoots of the apple tree. Some interesting variations are found among the flavonol compounds ; for example, in very young leaf kaempferol, in the form of a diglycoside, is the more important, while in mature leaf quercetin predominates, almost entirely as monoglycosides. In leaf of all ages phloridzin is the major phenolic, but a diglycoside of phloretin occurs only in young leaf.

The previously reported dihydrochalcone glucoside isolated from the leaf of *Malus trilobata* appears to be unique to this species, in which it replaces normal phloridzin. It has now been shown unequivocally to be the 4-glucoside of phloretin : phloridzin is the 2-glucoside. Because of conflicting statements in the literature about the occurrence of phloridzin and related compounds in plants, a check of the various reported occurrences is being made by chromatographic methods which were, of course, not available to the earlier workers. No phloretin derivatives have been found in any of the *Pomoideae* outside the genus *Malus*, nor in *Smilax* or *Prunus* species in which their presence had been claimed. Contradictory reports about the presence or absence of phloridzin and asebotin in the leaf of *Kalmia* and *Pieris* have been resolved by the observation that there is a clear-cut distinction between certain species. Thus *Kalmia latifolia* contains phloridzin, *K. angustifolia* asebotin, *K. polifolia* neither. Similarly most samples of *Pieris japonica* contain only phloridzin, and of *P. taiwanensis* only asebotin ; some garden varieties have both, and *P. floribunda* has neither.

See also Nos. 11, 62 and 63 in *Publications, 1960*, pp. 47-50.

Entomology.

Biology of Insect Pests.

Population counts of leaf hoppers, begun in 1958 on the cider varieties Sweet Alford and Stoke Red and the dessert variety Laxton's Superb, have shown that there are large fluctuations from year to year within the various species. Morphological characters of the nymphs have been determined for the identification of species. Work on the biology of the Willow Beetle, *Cryptorrhynchus lapathi*, has been continued preparatory to further experiments on control measures in the field.

Ecological surveys of insect pests of fruit crops, particularly of the black currant, are being made. Besides the usual biological information, records of habitat grouping are being kept ; specimens are collected as community groups. The Glasshouse Red Spider mite (*Tetranychus urticae*), which has become much more prevalent on black currants because of the increased use of sprays and the lack of predators, is receiving special attention.

Spray Techniques.

In further work, on the formulation of sprays, the effect of the surface properties of the spray liquid/solid combination on the retention of sprays has been examined by studying the sliding of liquid drops on solid surfaces. A theory has been evolved to explain the movement of drops and to predict the volume of spray liquid that may be retained on a solid surface. The retention

may be expressed in terms of the air/liquid surface tension, the advancing and receding contact angles of the liquid on the solid surface, the angle of tilt of the surface and the density of the spray liquid; the influence of the spray droplet spectrum on retention may be covered by the use of a factor which is a constant for a given spray spectrum. The retention found in practice agrees with the theoretical figure for most spray/solid combinations under comparable conditions of spray droplet impaction; the effect of the velocity of the impacting droplets is a complex problem which is still under consideration. It appears possible to calculate a retention factor for any spray liquid/solid combination; by a few simple laboratory tests, the relative retention efficiencies of different spray formulations may then be assessed. A large range of surface-active agents is now being studied in this way.

A critical survey has been made of the reliability of the Flying Spot Particle Resolver for the automatic measurement of microscopic particles. The speed and accuracy of automatic measurement were found considerably greater than those achieved by visual assessment with a standard optical microscope. Basically the instrument is very accurate, but the accuracy with which a particular sample is assessed largely depends on the nature of the sample. Inaccuracies are introduced by overcrowding or aggregation of the particles on the sample, by irregular-shaped particles, and by individual particles having poorly-defined edges.

In field trials on the control of apple aphids both pyrethrum (0.005% pyrethrins) and a DDT/BHC emulsion (0.025% DDT; 0.007% BHC) with a succinate wetter (0.025%) gave good control of the Oat-Apple and Rosy Apple aphids when applied by large-volume run-off methods at the 'mouse-ear' and 'green cluster' stages; both materials failed when applied by small-volume spraying methods at the same concentrations and also at five times the concentrations. Malathion (0.05%), which gave virtually complete control when applied in large volume, also failed to control the aphids when applied in small volume, but gave control when the concentration was increased five times; as in previous years, at the low concentration of 0.025% it gave excellent control of aphids at the 'green cluster' stage when applied in large volumes with added wetting agent. Demeton-methyl (0.0375%) gave complete control in large volume and good control also when applied as dilute material in small volume.

Using fluorescent tracers it was shown that in the large-volume applications the spray liquid had penetrated between the flower stalks within the cluster where the aphids were then situated: by contrast, the spray liquid of the small-volume applications was deposited only on the leaves and the outside of the blossom buds. Small-volume deposits of demeton-methyl and concentrated malathion therefore appear able to control the aphids from this position, whereas pyrethrum, DDT/BHC and dilute malathion need to penetrate between the flower stalks (p. 89).

In field trials, large-volume hand lance spraying with phenkapton (0.02%) gave excellent control of the Fruit Tree Red Spider mite, *Metatetranychus ulmi* (Koch), when applied at the 'petal-fall' stage. A similar application of chlorbenside (0.02%) failed to control the mite.

In 1959 it was shown that six drenching applications of 1% lime sulphur with succinate wetter, at fortnightly intervals from the grape stage, gave excellent control of the Black Currant Gall mite. In 1960, different methods

of giving six applications of 1% lime sulphur at 200 gallons and 20 gallons per acre were tested. Large-volume hand spraying gave excellent control, and automatic spraying with a vertical mast was only slightly less effective; large-volume applications by compressed-air nozzles were slightly more effective than applications by overhead boom. Small-volume applications of 1% lime sulphur by compressed-air nozzles were ineffective: small-volume applications of neat lime sulphur by compressed-air nozzles gave good control of the mite but damaged the bushes badly (p. 129).

Population studies have shown that whilst the complete eradication of the mites demands chemical protection of new growth from the "early grape" stage until July, good control can be obtained by three hand-lance applications of 1% lime sulphur; one at the early grape stage, one in the blossom period and one in the post-blossom period. In preliminary experiments $\frac{1}{2}$ % lime sulphur has appeared as effective as 1% (p. 124).

Work has continued on the behaviour of fluorescent tracers at the lower limits of visibility. The effect of relative humidity on the evaporation of spray droplets of various sizes is being studied to determine the change in visibility likely to result from the increased concentration of tracer in the droplets.

Both fluorescent tracers and chemical assays of copper were used in North Borneo to evaluate the relative performances of hydraulic sprayers and knapsack mist-blowers in applying copper fungicides for the control of Frog-eye disease of cigar-wrapping tobacco. When used at the correct walking speed the mist-blower was found to give a satisfactory cover on both surfaces of the leaf. An application of copper oxychloride (0.1% Cu) with 0.025% wetter (Ethylan C.P.) gave 0.2 μ g. Cu/sq. cm. on the upper surface and 0.4 μ g. on the under surface.

At Long Ashton deposits of copper on apple leaves sprayed to full coverage were found to vary with the formulation of the fungicide: the copper deposit from Bordeaux mixture was twice that obtained from a finely divided copper oxychloride containing the same concentrations of copper and wetter.

Work on the application of DDT to coconut spadices by means of a hand canister pneumatic sprayer was continued in collaboration with the Kenya Department of Agriculture. It was found that after seven days the residual deposit was no longer toxic to the Nutfall Bug, *Pseudotheraptus*.

Spray Machinery.

Manually operated compressed-air spray equipment was designed for use in a spray chamber required for the application of pyrethrins for the control of tsetse fly resting on vehicles, domestic animals, baggage, etc. The equipment was specially designed for use by African labour.

Investigations on the production of low-volume mist-blower spray nozzles were continued and a promising design was incorporated in a tractor-mounted machine. The machine is of simple and robust construction and aims at producing a close spectrum of small droplets by means of air shear of the liquid. It requires 18 h.p. to drive the centrifugal fan and is capable of atomising up to 25 gallons per minute: increases in throughput are accompanied by an increase in droplet size.

An air nozzle was designed to improve the distribution of droplets formed by a knapsack power mist-blower; this special nozzle was required for spraying tobacco.

Plant Pathology.

mostly collected

Field observations showed that strawberry mildew (*Sphaerotheca humuli*) persisted on the plant as superficial mycelium on the overwintering green leaves. In spring this mycelium produced conidia which germinated on leaf surfaces over a range of 2°–35°C and from 0 to 97% relative humidity (optimum 20°C and 97% r.h.). Most conidia were killed by a few hours immersion in water.

S. humuli on strawberry could not be transferred to hop : it would infect *Potentilla fragariastrum* under glass but not in the field. No strawberry varieties were immune to mildew : the most resistant were Huxley, Cambridge Favourite and Late Pine. Surface waxes from strawberry leaves stimulated germination of *S. humuli* conidia on glass slides, but other cuticular fractions showed inhibitory effects.

In trials at Luddington, unsprayed plots yielded 83% (by weight) of mildewed fruits. Three sprayings with lime sulphur plus thiram reduced this to 15% ; two sprayings to 30%. Thiram plus dinocap or thiram alone gave no significant control of mildew in 1960. Tasting of the canned treated fruits gave inconclusive results, but fruits that had received two thiram sprays were generally disliked.

In experiments on the control of *Nectria* canker on Worcester Pearmain, two autumn applications of Bordeaux mixture (0.25% Cu) followed by one spring application significantly reduced the incidence of leaf-scar cankers. Stripping of foliage on a dry day in advance of leaf-fall diminished the number of infections resulting from leaf-scar inoculations at the normal leaf-fall period : experiments with chemical defoliants showed that dipyriddy (0.12%) and Bordeaux mixture were fairly effective.

Lesions on apple shoots caused by *Gloeosporium perennans* developed most rapidly and spored most freely when atmospheric and soil moisture levels were high.

Preliminary results of fruit infections with spores of *G. perennans* showed that Worcester Pearmain fruit from clover plots was more readily rotted than that from arable plots ; that fruit from perennial-rye plots rotted as quickly as that from "tumble-down" plots, and that the timothy plots gave the best-keeping fruit. The application of extra nitrogen appeared to reduce rotting in this instance, both on Worcester and Cox fruit.

A proprietary phenyl mercury compound, used at 0.1% Hg as a winter spray on trees of Cox, considerably reduced the level of rotting on the fruit crop of the following season. Comparable trials carried out yearly since 1957 have shown that the reduction obtained has been influenced to a large extent by the season, indicating a loss of fungicidal effect following increased rainfall. This has been confirmed by the analysis of bark and washings from trees treated with mercury compounds and then subjected to known rainfall. The percentage germination of spores of *G. perennans*, in contact with treated bark, rises rapidly when the residual mercury falls below a certain level.

Two pre-cropping applications of zineb reduced black currant leaf spot by more than 25% compared with control ; captan gave a reduction of about 2%. Leaf spot developed more rapidly than in 1959 on both Baldwin and Mendip Cross, Baldwin again being more readily infected. Both varieties showed in 1960 an improvement in crop weight on all plots sprayed in 1959.

Studies were continued on the formation of extra-cellular polygalacturonase by *Sclerotinia fructigena* (the brown-rot fungus). Production of the enzyme could be induced by D-galacturonic acid within 1 hour. The action was inhibited by actidione, 5-fluoro-uracil, *p*-fluorophenylalanine and 1% glucose. The polygalacturonase produced by the fungus could be inactivated by oxidized polyphenols from tea. Both epigallocatechin gallate and its oxidation products were similarly effective, but not epigallocatechin.

Tests were completed of the fungistatic activity against *Aspergillus niger* of a series of 6-alkyl-8-hydroxyquinolines. Oxalic acid secretion by the fungus was important in modifying the toxicity of metal chelates of these compounds : high concentrations of the acid chelated preferentially with the metal, liberating the free alkyl hydroxyquinoline.

See also Nos. 8, 10, 11, 20, 31, 36 and 58 in *Publications, 1960*, pp. 47-50.

Cider and Fruit Juices.

Organic Constituents.

As a result of the abnormally high specific gravities observed in dry perries of the 1959 season a study has been made of the "non-fermentable" substances in perries and ciders. The contributions made by sorbitol, leucoanthocyanin, malate and citrate to the residual gravity have been determined (p. 135). In the course of this work it was found that pear leucoanthocyanin is strongly dextro-rotatory (p. 140).

During work on sulphite fixation in ciders (p. 144) it was shown both in ciders and model systems at pH 3.1 and 4.1 that, in the presence of sulphur dioxide, dehydroascorbic acid is converted quantitatively into L-xylosone. In the absence of sulphur dioxide, dehydroascorbic acid in the model systems yielded no xylosone ; it is possible that xylonic or lyxonic acids, or both, were formed.

The addition of 1-2 mg. of thiamine per gallon to high-nitrogen juices reduced the amount of pyruvic acid formed during fermentation : little or no synthesis of malic acid occurred in these fermentations. In low-nitrogen juices malic acid was synthesised but very little pyruvic acid was found ; on the addition of asparagine much more pyruvic acid was formed than when an equal amount of ammonium sulphate was added, but in neither fermentation was malic acid synthesised. An adaptation of a method using *Lactobacillus arabinosus* 17-5 was used for the determination of malic acid.

The volatile compounds contributing to the flavours of ciders and perries have been further examined both chemically and by gas chromatography ; the effects of fermentation techniques upon these components are also being studied. The main differences noted between ciders and perries are that ciders contain greater amounts and a wider range of higher alcohols and esters than perries. In perries, and in pear juices, acetaldehyde and ethyl acetate are present in higher concentrations. The addition of a synthetic mixture of the main volatiles found in perry to a perry deprived of volatiles gave a reasonable imitation of the original product, but the finer points of flavour appear due to components present in traces and as yet uncharacterised.

In perries the amount of acetaldehyde was found to be greater after fermentation in the presence of sulphur dioxide, as would be expected. In ciders, the fermentation of depectinised juice resulted in some loss of flavour that appeared related to a lower content of fusel oils.

Metallic Complexes.

Further work on iron complexes of fruit components was carried out during a study period with Professor Schwarzenbach in Zurich; ferric and ferrous complexes of catechol, caffeic acid, chlorogenic acid, quinic acid and malic acid were studied by potentiometric and oxidation-reduction potential measurements. Complex formation between ferrous iron and the phenolic compounds appeared to be very weak but could not be investigated fully since, at pH values approaching neutrality, rapid oxidation to ferric complexes occurred. The ferric complexes of the phenolic compounds were strongly coloured and contained up to three moles of phenolic per atom of iron. Quinic and malic acids formed only weak ferrous complexes; the stronger ferric complexes of these acids were more fully investigated. The ferric quinate complexes were mononuclear over the pH range studied (2-4) and possible structures were indicated. The ferric malate complexes were mononuclear at pH 2 but, with increasing pH, dinuclear complexes were formed and predominated at pH 3.

A rapid method for determining iron in ciders and related products is described on p. 147.

Unfermented Juices.

The development of unfermented apple juice as an outlet for the lower grades of commercial fruit has continued. Tests have been carried out on behalf of potential manufacturers and, following the tests previously reported, commercial production is in progress. Demonstrations of beverages based on apple juice have been continued: an exhibit at the Wisbech Fruit Show and Marketing Exhibition in 1960 illustrated the use of cans, bottles and automatic vending in cartons as methods of distribution. Further work is now in progress in collaboration with the Horticultural Marketing Council.

Sulphur Dioxide in Cider-making.

Sulphur dioxide is used to control the fermentation of cider; it is also commonly added to cider at the time of bottling as a safeguard against chance infection. The cider must retain enough free SO_2 after bottling to enable the organisms to be killed.

For a given total SO_2 content, the equilibrium free SO_2 content of a cider is determined by the concentration of the different SO_2 -binding constituents and by the dissociation constants of their bisulphite compounds. Acetaldehyde is quantitatively the most important SO_2 -binding constituent of cider; an improved method for its determination has been devised and the dissociation constant of acetaldehyde bisulphite has been determined. Other SO_2 -binding compounds include pyruvic acid, α -ketoglutaric acid and xylosone. Glucose combines only weakly with SO_2 , to the extent of about 10% of the free SO_2 content of the cider.

The effect of different fermentation treatments on the SO_2 -binding power of cider produced from concentrate has been investigated. Fermenting in the presence of SO_2 did not greatly affect the SO_2 -binding power, but adversely limited the amount of SO_2 that could legally be added to the finished cider. Ciders of lower SO_2 -binding power were obtained by (a) adding thiamine before fermentation and (b) fermenting at high original gravity and diluting

the cider after fermentation. These differences were reflected in, but not quantitatively explained by, differences in the pyruvic acid and α -ketoglutaric acid content of the ciders. The two ciders with the highest SO_2 -binding power—low original gravity and no addition of thiamine—permitted yeast growth after sulphiting and bottling.

In conjunction with this work, the lethal effect of sulphur dioxide on individual yeast species has been studied. The LD_{50} values, or the amounts of free SO_2 or of undissociated H_2SO_3 required to kill 50% of a culture within 6 hours of inoculation, are as follows:—

Saccharomyces uvarum = 18 p.p.m. free SO_2 at pH 3.0 or 1 p.p.m. H_2SO_3

Saccharomyces cerevisiae = 24 p.p.m. free SO_2 at pH 3.7 or 0.33 p.p.m. H_2SO_3

Saccharomyces ludwigii, sometimes found as an infection in bottled ciders, was found able to resist 77 p.p.m. free SO_2 at pH 3.7, and therefore to survive in a cider containing more than the statutory limit of 200 p.p.m. total SO_2 .

Ciders with low sulphite-binding capacities, produced from concentrates by the methods described above, have been proved capable of destroying infections of *Saccharomyces* spp.

Fermentation of Concentrates.

Two properties of apple juice concentrates are being examined: their ability to support the growth of cider yeasts and bacteria and their fermentability after dilution.

Pure yeast cultures were added to concentrates differing in specific gravity, pH and state of oxidation. A large proportion of all the yeasts tested were killed after momentary contact with the concentrates. The remainder survived for about a week but thereafter only osmophilic yeasts remained viable; even these died out within three months in concentrates whose specific gravity was greater than 1.250. It would therefore appear that diluted concentrates should always be fermented with an added yeast culture.

The survival of selected cider bacteria was studied in juice concentrated sixfold. Oxidised concentrates killed the organisms rapidly, reducing their numbers to a very low level within five minutes of inoculation. After one week's storage the concentrates were sterile. Concentrates in which a minimum of oxidation had occurred were less lethal; although the number of organisms dropped considerably immediately after inoculation, a few survivors could be isolated from low-acid concentrates after a week's storage at 5° or 15°C. In contrast, high-acid concentrates were sterile after the same period of storage.

Fermentability has been assessed by the rate of loss of CO_2 from yeasted diluted concentrates, in which the effects of heating during concentration, and of storage conditions, are being studied.

Metabolism of Malate and Citrate by Lactic Acid Bacteria.

A heterofermentative cider isolate resembling *Lactobacillus brevis* has been examined for its enzymic activity towards malate and citrate by measuring CO_2 output in a Warburg apparatus at 35°C.

Washed cells grown with glucose and citrate showed considerable activity towards citrate in the presence of KCl, arabinose, Mg^{++} and Mn^{++} (Q_{CO_2} = 58 $\mu\text{l.}$ per hour per mg. dry weight of cells, reduced to 26 $\mu\text{l.}$ in the absence of arabinose). The optimum pH of the enzyme system using washed cells was 6.5.

Cells grown without citrate showed virtually no activity towards citrate but, after one hour's incubation with citrate, cells grown in the presence of glucose or glucose and malate showed slight activity. The metabolism of citrate seems therefore dependent on an induced enzyme.

Cells grown with malate and glucose showed active decarboxylation of malate in the presence of KCl. Arabinose, glucose and Mn^{++} stimulated the process: Mg^{++} did not. Cells grown without malate showed less active decarboxylation of malate, with no detectable induction period; in the presence of Mn^{++} , KCl and acetate buffer pH 4.5, $Q_{CO_2}=51 \mu l.$, compared with $117 \mu l.$ for cells grown with malate. Both arabinose and glucose accelerated decarboxylation. The optimum pH for malate decarboxylation by cells grown in the presence or absence of malate was 4.0–4.5, compared with 4.5–5.0 for *Lactobacillus arabinosus* 17–5. It seems likely that malate metabolism in the cider isolate is largely brought about by a non-adaptive enzyme system, probably by malic dehydrogenase and oxaloacetic decarboxylase. The difference between the enzyme systems of the two organisms was further shown by the greater instability of that of the cider isolate on storage, and by its inhibition by malonate.

General Microbiology.

Work on the mutation of acetic acid bacteria (Report for 1959, p. 37) has been extended to other genera of bacteria. Of eleven randomly selected bacterial strains, representing ten genera, all cultures were found to contain more than one colonial form. Some of these morphological differences within a culture could be linked with differences in biochemistry, thus suggesting that the phenomena observed in acetic acid bacteria are more widespread than noted hitherto (15).

As a result of these observations, a culture of the slime-forming cider lactobacillus was studied in detail. This culture, which had been maintained for some 3 years by weekly sub-culture, differed in several respects from the freshly isolated strain. Furthermore, several colonial forms were isolated from this culture, and they in turn differed from one another in such properties as the ability to produce slime and to ferment various sugars. These observations suggest that the lactic acid rod most commonly found in ciders—*L. pastorianus*—may well be a mutant of the slime-forming organism, since there is little difference between it and the non-slime-forming mutants derived from the slime-forming strain.

Methods for differentiating organisms of the genus *Acetomonas* from those of the genus *Pseudomonas* which bear a superficial resemblance to one another have been devised and published in collaboration with Dr. J. L. Shimwell of British Vinegars Ltd., and Dr. Muriel E. Rhodes of the University of Reading (18).

The nutrition of a strain of *Acetobacter aceti* has been studied with a view to its use in different methods of vinegar manufacture. The virtue of this species is its ability to use ammonium salts or other simple forms of nitrogen, with ethanol as sole carbon source. Tests showed that certain amino-acids substituted singly for ammonium sulphate (all levels of nitrogen being the same) were superior to the latter in supporting the growth of this organism. These were, in descending order of efficiency, γ -aminobutyric acid, glutamic acid and proline.

Pilot Plant.

During the year the section has been helped by gifts or loans of equipment, including a fully instrumented climbing-film evaporator, a pressure fermentation vessel, and a heat exchanger. Advice has been given to companies developing machines for extracting juice continuously, and models will be tested as they become available.

See also Nos. 3, 9, 12, 13, 14, 15, 16, 17, 18, 32, 48, 49, 55, 56, 59, 60 and 61 in *Publications*, 1960, pp. 47-50.

Domestic Food Preservation.

The increasing use of domestic deep freezers has emphasized the need for studies of the microbiology of frozen foods. Although much work has been carried out on commercially frozen products, no information is available on a domestic scale, where different working conditions and sources of contamination give rise to a widely different initial flora.

Bacteriological analyses of deep-frozen vegetables are being made at all stages of processing and freezing, to determine both the total microbial population and the relative numbers of important genera. The effects of various times of freezer storage, of subjecting the foods to cycles of thawing and re-freezing, and of thawing them by the different methods likely to be used in the home, will also be examined. It is hoped that besides giving information on the basic flora of fresh and frozen vegetables, and the possible presence of pathogenic species, the experiments will indicate the stages in processing at which the numbers of organisms may increase, and the precautions needed to minimise health risks.

The changes in the ascorbic acid contents of vegetables caused by deep-freezing and canning under domestic conditions have been further investigated. Quality assessments of colour, texture and flavour of preserved vegetables have again been made. Thirty-five varieties of vegetables were tested and a further twenty-four varieties preserved for testing during the coming year. The results for varieties tested for three years are given on p. 155.

Fruit varieties preserved for subsequent tasting include apples, blueberries and strawberries received from the National Fruit Trials, Faversham, and from N.A.A.S. trials at Wareham and Westbury-on-Trym respectively. Apples were preserved by bottling and freezing as pulp and slices and were also tasted both stewed and baked.

Work has continued on taint due to spray chemicals. For the Plant Pathology section, strawberries sprayed with thiram, dinocap and lime sulphur have been tasted after bottling and freezing; for the Entomology section, black currants with various lime sulphur treatments have been preserved by bottling and freezing, and as jam and syrup, to be tasted after 6 months' storage at room temperature.

Covers for tomato sauce and mushroom ketchup have been investigated. These products require processing after bottling to ensure keeping quality, and in the past waxed corks have been used in conjunction with screw caps. It has been found that screw caps of lacquered metal with ceresin inner disc, or plastic caps with or without waxed cardboard disc, fitted loosely during processing and subsequently tightened, are sufficiently airtight to prevent spoilage without the use of a cork.

The replacement by glucose of 50% of the sucrose (cane-sugar) in syrup-packs of frozen loganberries and blackberries gave slightly better colour to the fruit, but all-sucrose syrup was generally preferred for flavour. In dry packs, there was little difference in flavour between all-sucrose and sucrose-glucose, but all-sucrose was much preferred for appearance.

Willows.

Variety Collection.

Four fast-growing hybrids of *Salix alba* × *S. fragilis* were received from Germany and three selected varieties of *S. triandra* from Holland, bringing the total number of varieties in the collection to 203. Two new beds were laid out to incorporate 44 of the 61 varieties received from Scotland in 1954.

Experimental Work.

A replicated experimental plot of *S. triandra* Black Maul, employing five different spacings and three methods of planting, was established at Long Ashton to study the effect of spacing and depth of planting on annual yield and quality.

Preliminary tests at Long Ashton (Report for 1959, p. 39) showed that a good control of grasses in willow beds could be obtained with dalapon. In mid-May dalapon was applied on a field scale in Somerset at 7 and 14 lb./150 gallons per acre. Neither rate damaged the willows or had a significant effect on the number or length of shoots. Counts made in July showed that the area occupied by grasses had been reduced from 63% in control plots to 5% and 3% in plots receiving 7 and 14 lb. dalapon/acre respectively. Despite a proportionate increase in herbs, notably *Ranunculus repens* and *Potentilla anserina*, no weeding was necessary.

A test of residual herbicides at Long Ashton showed that simazine and monuron, at 4 lb./100 gallons/acre, controlled annual weeds in a newly planted willow bed without damage to the willows; the same rate of application of dalapon and 2,4-DES gave a less satisfactory control of weeds.

Studies of the etiology of willow rust, *Melampsora amygdalinae*, made in collaboration with the N.A.A.S., were continued on five willow beds varying in age from one to seven years. The early season was again unfavourable to rust development and, as in 1959, the mean number of cankers was only 0.11 per rod; but from July wet weather encouraged the disease and in October the mean number of cankers in a heavily infested bed was 1.3 per rod compared with 0.02 in October of 1959. The quality in this bed was so poor that it would have been more economic to market it as "brown willow" than to process it into "buff".

In collaboration with the Workshops section two willow-cutting machines were tested at Long Ashton and in Somerset during the winter. Their performance was greatly aided by small, low stools; experimental plots have therefore been laid down to examine the effect of mechanical cutting on the shape and vigour of stools and on crop yield. Stools reduced to ground level in one set of treatments produced significantly fewer but longer rods in the following year.

Advisory Work.

There were 204 requests for information during the year. 74 visits were made as below :

	South- West	Home Countries	East Anglia	Midlands	North	Wales	Scotland	Foreign
Enquiries	38	70	16	8	16	3	7	46
Visits	33	2	0	0	1	0	0	38

Ten requests for planting material were received from research or educational centres, of which five were again from schools or institutions wishing to establish small beds to produce material for their Handicrafts section. A further 1,000 cuttings were sent to complete the Lancashire County Council amenity scheme begun in 1958. A selection of species was sent to Holloway College, London, for studies of the translocation of phenolic compounds, and to the Forest Research Station, Ilanath, Israel, where the production of rough baskets for the fruit industry is being investigated.

The wet season produced a heavy crop of rank willows of poor quality, with an abundance of cankers caused by Willow rust. Insect pests caused little damage ; the incidence of Willow beetle was particularly low. Flooding was very serious ; many moors were under water for three months and cutting was so delayed that it is doubtful whether all the year's crop will be harvested.

Interest in willow for the production of pulpwood and similar products has continued, but trials of *S. aquatica gigantea* first planted at Long Ashton in 1957 show that neither its rate of growth nor its habit is satisfactory for commercial production. Of six hardy selections supplied to the Forestry Commission in Devon (Report for 1959) *S. daphnoides* has again shown the greatest promise.

Since the report on the economic position of the willow-growing industry was made in December 1959 (Report for 1959, p. 155), the industry has improved ; there has been a small increase in demand and prices have remained steady at around 26/- per bundle of processed willows.

Information on the economic position in Holland, West and East Germany and Poland was obtained during a tour of centres of willow-growing and research made in September and October. Willow-growing and basket-making are predominantly manual occupations, and are declining in Britain, Holland and West Germany because the rates for labour cannot compete with those of modern industries. It is not likely that they will die out completely, because in Germany there will always be a luxury demand for fine fancy baskets ; in Holland willow-growing is associated with land reclamation and river defence work, while in Britain some demand for willows will always be forthcoming from Blind Asylums and other institutions.

Willow growing and basket making are prosperous and expanding industries in Poland because Poland has abundant cheap labour and can utilize poor sandy soils for which there would otherwise be little use. Poland's willows and baskets are sold at highly competitive prices in western markets and bring her valuable foreign currency.

In East Germany, the government has created a demand for rough baskets by decreeing that they shall be used by agriculture and industry in preference to other kinds of containers. Hence willows are being established to replace beds lost in partition or ceded to Poland ; they are grown on an extensive scale and a great effort is being made to mechanize methods of production and processing to counter the shortage of labour (p. 159).

Statistics.

Experimental Design and Analysis.

Advice and assistance to members of Station staff have again formed the largest part of the section's work. Other members of University staff have also consulted the section, and a general course of lectures for students in the Faculty of Science is being undertaken in the current academic year.

In collaboration with the Plantations section, several layouts have been prepared for the planting of experimental plots at Long Ashton, both for long-term top-fruit trials and for work on bush and soft fruits. Several trials of herbicides have also been planned and suitable methods of sampling and recording evolved.

Analyses of laboratory results have been made, particularly for the Plant Pathology and Physical Chemistry sections and for official committees. In collaboration with the Cider Section, experiments have been planned to check the precision of results obtained by using a number of different pipettes in cell-counting tests, and hence to select pipettes suitable for routine use.

Examination of girth measurements obtained for the variety Cox's Orange Pippin in the apple nutrition trial at Long Ashton (Plot 18) revealed a spurious nitrogen effect, due to uneven growth of the trees in the period after cover crops had been sown but before fertilizers were applied. The initial calibrating measurements taken in accordance with normal practice were not effective in allowing for this; a modified variate has been developed that allows the effect of covers to be distinguished from the natural variation.

Several years' data obtained in experiments on the nutrition of bulbs at the Kirton Experimental Husbandry Farm have been examined, in collaboration with the Plant Physiology section, for differences in the distribution of bulb size induced by nutritional status. Large effects due to nitrogen, and to initial bulb size, appeared.

Biometric Studies.

The examination of artificially-flavour-based tasting samples of the fungicides thiram, dinocap, captan and lime sulphur indicated the incidence and persistence of off-flavours in fruit preserved after treatment with these fungicides. Field trials in 1960 were disappointing, because of the poor quality and scarcity of samples of 'control' (unsprayed) fruit. In these trials, off-flavours due to fungicides were no more marked than those due to mildew in the controls.

A discriminant function method of using their anatomical features for differentiating between rootstocks was completed in collaboration with the staff of the computer at Rothamsted; of a number of clonal stocks examined, only Malling IX could be identified in this way, variability among the others being far too great to allow of valid discrimination.

Preliminary measurements have been taken in an attempt to relate vigour and cropping of raspberries grown in pots. A method of sampling and measuring shoots of black currant bushes for indications of vigour, advocated elsewhere, has been found impracticable at Long Ashton because of the profuse growth obtained.

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VIRUS DISEASES OF FRUIT TREES: VII. LATENT VIRUS IN APPLE VARIETIES AND ROOTSTOCKS.

By A. I. CAMPBELL.

Introduction.

The latent viruses present in some of the apple varieties and rootstocks growing at Long Ashton Research Station have been described by Luckwill and Campbell (1). A preliminary survey showed that many of the scions and rootstocks that had been tested and found free from apple mosaic, rubbery wood and chat fruit viruses, were infected with one or more latent viruses that gave rise to (a) line pattern; (b) dwarfing and (c) scaly bark, when inoculated into *Malus platycarpa*. It is uncertain how many different viruses or strains of virus are concerned in producing these symptoms, which can occur with varying severity in any combination, except that scaly bark does not occur without either dwarfing or line pattern.

In the present work the distribution of latent virus has been studied in clonal rootstocks, in dessert and culinary varieties, and also in cider varieties.

Experimental.

Clonal Apple Rootstocks.

Although it had been shown that many of the rootstocks in the Long Ashton stoolbeds were infected with latent viruses, little was known about the distribution of these viruses among other clones of rootstocks, particularly in those found free from apple mosaic and rubbery wood. Virus indexing by double budding was therefore started in 1958 with the co-operation of East Malling Research Station, who supplied many clones known to be free from apple mosaic and rubbery wood. Apple seedlings were budded with *M. platycarpa* at about 4 inches above soil level; two inches below this bud, and on the same side of the stock, the inoculation bud was inserted. The *M. platycarpa* bud was allowed to grow during the following season but the inoculation bud was rubbed out as soon as it had united with the rootstock. The subsequent growth and symptoms of *M. platycarpa* after inoculation with 5 buds of each clone are given in Table I.

It can be seen that all the clones of the early Malling series of rootstocks were infected with latent viruses. All clones of M.IV, M.VII and M.IX produced the three virus symptoms on *M. platycarpa*, but only one of the five clones of M.II showed dwarfing. The two clones of Crab C were free from scaly bark but all seven clones of M.XXV, a later introduction, were severely infected and showed all three symptoms.

The comparatively recent introductions, M.26, MM.104, and MM.111, were entirely free from infection, as were four of the five clones of MM.106; one was infected with line pattern only. The five clones of MM.109 tested, however, all showed the three virus symptoms.

A more detailed examination of the Long Ashton stoolbeds, carried out in 1959 on twelve stools of M.II, M.IX, M.XXV and MM.109, showed that while the general pattern of infection was constant, some stools appeared to produce more severe symptoms on *M. platycarpa* than others. No healthy stools were found during this survey: those that had been previously tested reproduced the same symptoms, particularly the degree of severity of dwarfing.

TABLE I.

SYMPTOMS AND HEIGHTS OF *MALUS PLATYCARPA* INOCULATED WITH CLONES OF VARIOUS ROOTSTOCKS.

Rootstock	No of Clones	Clones giving Virus Symptoms			Height (cm.)	
		Line Pattern	Scaly Bark	Dwarfing	Range	Mean
M.II	5	5	4	1	79-102	91
M.IV	3	3	3	3	41-68	53
M.VII	3	3	3	3	18-45	31
M.IX	3	3	3	3	33-54	43
M.XXV	7	7	7	7	28-88	48
M.26	3	0	0	0	101-119	112
MM.104	4	0	0	0	97-135	107
MM.106	5	1	0	0	94-139	118
MM.109	5	5	5	5	36-83	60
MM.111	5	0	0	0	107-135	116
Crab C	2	2	0	1	50-77	63
Control (uninoculated)	—	0	0	0	82-137	118

Dessert and Culinary Apple Varieties.

Dessert and culinary varieties tested at Long Ashton had been shown to be severely infected with the same viruses as the rootstocks (1). In 1959 the effect of inoculations from various clones of varieties on the symptoms and growth produced by *M. platycarpa* were studied. A similar technique to that described for the rootstocks was used. Four varieties, Bramley's Seedling, Cox's Orange Pippin, Winston and Worcester Pearmain, were obtained from two sources :

- A. Tested mother trees growing at Long Ashton and found free from rubbery wood and apple mosaic viruses.
- B. The oldest tree of the variety obtainable. The Bramley's Seedling scions came from the original unworked tree, now about 150 years old. Cox and Winston came from trees propagated once from the original seedling and about 60 and 40 years old respectively. The Worcester scions were taken from a tree about 70 years old.

Table II shows the symptoms produced on *M. platycarpa* after inoculation with the four varieties, and the average height of the indicator trees at the end of the growing season. Only trees exceeding 5 cm. in height were included in this average.

It is evident that, except in Cox, the severity of infection, shown by the average-height of the *M. platycarpa* indicator, is greater from the virus-tested source than from the very old trees. The material from the original Bramley's Seedling tree appeared to be still completely free from all known viruses. Winston, a relatively recent introduction, but grafted once, was infected only with line pattern virus.

Cox's Orange Pippin and Worcester Pearmain from both sources produced similar leaf and shoot symptoms on *M. platycarpa* but the height of the indicator was considerably lower after inoculation with the virus-tested clones of Worcester than after inoculation with the clones from the very old trees.

TABLE II.

SYMPTOMS AND HEIGHTS OF MALUS PLATYCARPA INOCULATED WITH CLONES OF DESSERT AND CULINARY APPLES.

Variety	Source	No. of trees	Line Pattern	Scaly Bark	Dead or very weak	Av. height (cm.)
Bramley's Seedling ..	A	15	10	7	4	56
	B	13	—	—	—	95
Cox's Orange Pippin ..	A	15	14	—	1	87
	B	14	12	1	2	84
Winston	A	15	12	4	3	67
	B	16	14	—	2	101
Worcester Pearmain ..	A	16	15	9	1	56
	B	15	14	3	1	89
Control (uninoculated)	—	11	0	0	0	109

Cider Varieties.

Scion material from cider varieties free from apple mosaic, rubbery wood and chat fruit viruses has been distributed from Long Ashton since 1958. These mother trees were re-tested during 1959 using the double budding technique with *M. platycarpa* as indicator.

TABLE III.

SYMPTOMS AND HEIGHTS OF *M. PLATYCARPA* INOCULATED WITH CIDER APPLE VARIETIES.

(Originally 6 trees of each variety).

Cider Variety	Number of Trees			Av. Height (cm.)
	Line Pattern	Scaly Bark	Dead	
Ashton Brown Jersey ..	3	2	3	37
Brown Snout	6	6	—	45
Bulmer's Norman	6	3	—	52
Fillbarrel	5	1	1	109
Kingston Black	3	—	3	112
Médaille d'Or I	3	4	1	40
Michelin	4	2	2	19
Stoke Red	6	6	—	87
Sweet Coppin	6	5	—	32
Tremlett's Bitter	5	3	1	71
Vilberie	5	4	1	78
Cider Seedling (A)	—	—	—	95
Cider Seedling (B)	—	—	—	108

Table III shows the symptoms and heights of *M. platycarpa* inoculated with eleven cider varieties and with two twelve-year-old unworked cider seedlings. It can be seen that in contrast to the seedlings all the named

varieties were infected with line pattern and all except Kingston Black produced scaly bark. Fillbarrel and Kingston Black had no dwarfing effect and vigour was only slightly reduced by Stoke Red, Vilberie and Tremlett's Bitter.

The distribution of latent virus among the cider varieties at Long Ashton appears to be very similar to that found amongst dessert and culinary varieties. Clones of cider apples from other parts of the West Country were found to be equally infected.

Discussion.

The presence of latent virus in so many clones in the original Malling series of rootstocks suggests that they became infected at an early stage, possibly before classification by East Malling Research Station. Infection may have been caused by the use of suckers or pieces of root from worked trees, or by the use of rootstocks that had been budded and failed to grow and had been returned to the stoolbed. Gilmer and Brase (5) have shown that once a small number of rootstocks become infected, the percentage of infected trees rises rapidly throughout the nursery when normal propagation methods are used.

It is difficult, however, to explain how the clones of MM.109 and M.XXV and one clone of MM.106 can have been infected; it is also strange that cider varieties that have been propagated on seedling stocks, usually considered to be virus-free, have become so severely infected. Although some transmission through root-grafting is possible, such a high level of infection suggests that another method of transmission may be involved. The fact that the original Bramley's Seedling tree is free from line pattern, scaly bark and dwarfing, after 150 years, would suggest, however, that infection by methods other than grafting is rare.

Little evidence is yet available to show what effect latent virus has on rootstocks and varieties. This was also true, however, of the effect of apple mosaic and rubbery wood on varieties tolerant to these viruses until recent work by Posnette and Copley (3) and Campbell (4) showed that both cropping and vigour could be affected. It therefore seems likely that clones of varieties and rootstocks that are free from latent virus will perform better than those infected, but until such clones are found or produced no comparison between healthy and infected can be made.

Recent work has shown that many of the varieties in the U.S.A. are also infected with latent viruses. The rootstock Spy 227 has been shown to be useful as a virus indicator, and a Russian seedling R12740-7A has been used by Mink and Shay (6) to show that a chlorotic leaf spot virus occurs in latent form in many American varieties. The relationship between these symptoms and those shown by *M. platycarpa* is at present uncertain. Unlike this country, however, many healthy clones have been found in the U.S.A. This may be due in part to the use of seedling rootstocks which, despite their many disadvantages, are usually considered to be virus-free.

Although the different clones of the varieties and rootstocks had varying effect on the vigour of *M. platycarpa*, no differences could be seen in their own vigour: the trees from heavily infected clones appeared as strong as those from healthier ones. It remains to be seen whether latent virus may affect factors other than vigour.

Acknowledgments.

Thanks are due to East Malling Research Station, particularly to Dr. A. F. Posnette and Mr. R. Copley who supplied many virus-tested rootstock clones ; to Dr. L. C. Luckwill for help and encouragement during this work, and to Mr. J. S. Coles and Miss Margaret Shellard for assistance in recording the experiments. I am also grateful to the N.A.A.S. officers who collected the scions from very old trees.

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THE EFFECT OF NURSERY TREATMENT ON THE GROWTH AND CROPPING OF BLACK CURRANTS.

By A. I. CAMPBELL and E. CATLOW.

Introduction.

It is general practice for nurserymen to sell certified black currants when they are two years old. The cuttings for these are usually taken in October or November from one-year shoots from other older nursery or stool bushes. The shoots are frequently cut into lengths of 6-8" and are often prepared indoors ready for planting in a narrow trench. Sometimes entire shoots are pushed into the soil to a similar depth and the top is then removed. The latter method requires greater care and a more thorough preparation of the site, and is not always possible because of the nature of the soil.

The treatment of the young bushes in the nursery also varies. Sometimes they are transplanted at the end of the first year, although in recent years most nurserymen have preferred to leave the bushes undisturbed during their two years in the nursery. Transplanting invariably produces a bush with a more fibrous root system and shorter shoots ; this is generally considered to facilitate the final transplanting and to promote growth in the following year.

These various nursery practices and their effects on the subsequent growth and cropping of the bushes have been examined ; notes were made of the times taken for the different operations.

Experimental.

NURSERY TRIAL.

Layout and Material.

Black currant cuttings were taken in October, 1955, from certified stool bushes of the variety Baldwin. The experiment compared the following four treatments :

- (A) Prepared cuttings, not transplanted.
- (B) Prepared cuttings, transplanted at end of first year.
- (C) Cuttings pushed into the soil, not transplanted.
- (D) Cuttings pushed into the soil and transplanted at end of first year.

Each treatment was repeated four times at random ; each plot contained 100 cuttings.

Cuttings 8" long were prepared from the basal part of current-year shoots and planted with the top bud 1" above soil level in rows 3' apart, with 6" between cuttings in the row. The cuttings made from entire shoots were pushed into the soil at the same spacing and to a depth of approximately 8". The top was then cut off 1" above soil level.

At the end of the first year of growth the bushes in treatments B and D were transplanted to 6" apart in the row. Bushes of all treatments were then cut down to within 2" from ground level.

Records.

At the end of the first year a count was made of the cuttings rooted, and the weight of the prunings was measured ; note was also made of the time taken to prepare and plant the cuttings. At the end of the second year the weight of the shoots and roots of a sample of the bushes was measured and the time taken to lift the bushes was noted.

Results.

There was no significant difference between the two methods of planting, either in the percentage of cuttings rooted or in vigour as expressed by the weight of prunings (Table I). As would be expected, the use of whole shoots was quicker than that of prepared cuttings.

TABLE I.
NURSERY TREATMENT : FIRST YEAR.

Treatment	Prepared		Not Prepared	
	A	B	C	D
Time to prepare and plant 400 cuttings (hr.)	5	5	2	2
Per cent rooted	76	69	78	66
Mean wt. of prunings (g.) from each bush	9.7	10.6	10.8	11.3
Transplanting and/or pruning (hr.)	$\frac{1}{2}$	6	$\frac{1}{2}$	6

During the second year the transplanted bushes grew less vigorously : Fig. 1, Plate I, shows a typical bush of each type at the end of two years. It can be seen that the transplanted bush has smaller shoots but a more fibrous and larger root system than the bush not transplanted. No significant difference was observed between the two types of cutting, hence only the effect of transplanting was subsequently considered.

Table II shows the mean weight per bush of the roots and shoots of a random sample of 24 bushes of each type when lifted at the end of the nursery trial. It can be seen that the transplanted bushes were easier to lift and handle, but that the number of bushes with three or more shoots 18" or longer was considerably less ; in addition the weight of shoots was only half that from the non-transplanted bushes.

TABLE II.
NURSERY TREATMENT : SECOND YEAR.

Treatment	Transplanted	Not Transplanted
Mean wt. of shoots per bush (g.)	51	114
Mean wt. of roots per bush (g.)	241	218
Time to lift 400 bushes (min.)	80	120
Number of bushes with three or more shoots >18" long	224	386

FIELD TRIAL.

Layout and Material.

The bushes in this trial were planted as part of a larger experiment on the control of leaf spot (*Pseudopeziza ribis*, Kleb). The whole trial contained 1,020 bushes at 8' x 3', in a split-plot randomized block design, covering 0.6 acres and planted in a medium loam on a south-facing slope. Of the six blocks of the leaf spot trial only four were used for this experiment. Each contained 3 main plots, split into two rows of 15 bushes each for the transplanted and non-transplanted treatments. The experimental bushes were chosen at random from those that had three or more shoots of at least 18".

Management.

The bushes were cut to ground level after planting in 1957/8. Pruning in the following seasons was determined by the desire to leave only strong one-year shoots for cropping. In the first two years after planting, one third of the current year's growth was cut to ground level ; in addition all small shoots were removed. In 1960 pruning followed commercial practice, about one-quarter of the bush being cut out.

After planting, a straw-mulch of 4 tons per acre was applied ; further mulches of 2 tons per acre were added in each of the following two years. Side dressings of complete fertilizer supplying 46, 84, and 115 lb. of N per acre were applied in 1958, 1959 and 1960 respectively. A normal spray programme was adopted except that no attempt was made to control leaf spot.

Records.

Vigour was assessed in the first two seasons by counting shoots over 12" long and in 1960 by measuring all one-year-old shoots over 8". Two crops were taken during the period and the weight was recorded in lb. per plot.

Results.

The results are shown in Table III.

TABLE III.
VIGOUR 1958-60 AND CROP YIELD 1959 AND 1960.
Mean per plot of 15 bushes (12 replicates).

		Transplanted	Not Transplanted	Sig. Diff.
No. of shoots	1958	58.0	66.5	7.60 (5%)
	1959	124.3	145.3	8.98 (1%)
Shoot length (ft.)	1960	334.6	452.8	58.17 (0.1%)
Crop (lb.)	1959	14.6	18.2	1.40 (1%)
	1960	41.8	51.2	1.77 (1%)

Growth has been satisfactory except in a few bushes and in a small area on the south-west exposed corner. It will be seen that the bushes not transplanted have always been significantly more vigorous than the transplanted ones and have become increasingly so. Fig. 2 shows a typical bush of each type at the end of the third season in the field. It can be seen that the bush not transplanted has more branches and that the roots arise largely from the crown of the bush, whereas the main roots of the transplanted bush arise at a lower level.

As would be expected, the yields followed a similar trend to the growth, and were significantly different in both years. In the second year the non-transplanted bushes yielded the equivalent of 2.75 tons per acre, half a ton per acre more than the transplanted ones. From the growth figures for 1960 it seems likely that this difference will continue for at least another year.

Discussion.

Since the unprepared cuttings rooted as well as the prepared ones and gave the same type of bush, the extra time needed for preparing cuttings would seem to be justified only in bad weather conditions or when the soil is unfavourable.

There is clearly no advantage to be gained from transplanting the bushes in the nursery: the results suggest that this practice is harmful rather than beneficial, for besides involving extra labour it gave a lower number of saleable bushes. A fibrous root system seems not over-important in a plant so easily rooted as the black currant. It is possible, however, that if the season after planting in the field had been a drier one, the difference between the transplanted and the non-transplanted bushes would not have been so great.

The roots of the two types of bush arise at different points. In the transplanted one there are two tiers, a lower one produced by transplanting in the nursery and a higher one developed in the field. It would appear that the surface roots, which are more abundant in the non-transplanted bush, are of

PLATE I.



Fig. 1. 2-year-old black currant bushes.
Left. Not transplanted. Right. Transplanted.



Fig. 2. 5-year-old black currant bushes.
Left. Not transplanted in the nursery. Right. Transplanted in the nursery.

greater value under straw mulch conditions in picking up available nutrients. The extra crop of $\frac{1}{2}$ ton per acre obtained in the second year stresses the importance of planting vigorous young bushes.

Acknowledgments.

We are indebted to many members of the Plantations, Statistics and Photographic Sections for their assistance in this work.

THE TOLERANCE OF FRUIT CROPS TO SOME RESIDUAL HERBICIDES.

By J. CASELEY.

Introduction.

Some of the newer residual herbicides, particularly the substituted ureas (e.g. monuron, diuron, neburon) and the substituted triazines (e.g. simazine, atrazine), show great promise as weed control agents in fruit crops. Applied to clean soil after the spring cultivation at rates of the order of $\frac{1}{2}$ to 6 lb. per acre, such herbicides can give effective weed control over periods of many months; there are reports of their successful use in black currants, gooseberries, raspberries and strawberries, in tree fruit nurseries and stoolbeds, and around the bases of established orchard trees.

The selective action of these herbicides is thought to depend chiefly on the fact that they are held in the top few inches of soil and do not normally penetrate to the root zone of the crop. However, their persistence in the soil for such long periods raises the question whether they may not eventually be leached to lower horizons and be taken up by the roots of the fruit plants, possibly many months after their application to the soil. This danger is greatest under conditions of high rainfall on soils low in organic matter, and with relatively shallow-rooted fruit crops such as strawberries and black currants. Should the herbicide be taken up by the roots of the crop, the extent of the resulting damage will depend on the innate tolerance of the crop to the particular chemical used; this is thought to be determined chiefly by the rate at which the chemical can be broken down in the plant tissues to non-toxic derivatives (1).

The preliminary experiments reported below were undertaken to assess the type and extent of damage likely to occur in the field in the event of herbicides of this type being taken up by the roots of various fruit crops. In order to ensure that the herbicides reached the roots, the experimental plants were grown in sand cultures to which the herbicides were added at the highest rates likely to be used in commercial practice.

Although the chief path of entry of the substituted urea and triazine herbicides is undoubtedly through the root system, it was not known whether sufficient of the chemical might be absorbed through the leaves to cause damage. This is an important practical consideration since it might be very difficult to apply the herbicide to the soil surface without wetting the foliage of low-growing crops such as strawberries and apple rootstocks. For this reason a second series of experiments was carried out in which the herbicides were sprayed on to the foliage of a variety of fruit crops at relatively high rates, and observations were made of the type and extent of the resulting damage.

Experimental.

Herbicides Used.

Commercial preparations of the following herbicides were used in these experiments :—

<i>Common Name and % active ingredient</i>	<i>Chemical Name</i>	<i>Water solubility (p.p.m. at 20°C)</i>
Amiben 50%	3-amino-2,5-dichlorobenzoic acid	700
Diuron 85%	N-(3,4-dichlorophenyl)-N'-N'-dimethylurea	42
Monuron 85%	N-(4-chlorophenyl)-N'-N'-dimethylurea	230
Neburon 50%	N-(3,4-dichlorophenyl)-N'-butyl-N'-dimethylurea	4.8
Simazine 50%	2-chloro-4,6-bis (ethylamino)-1,3,5-triazine	3.5

Method.

The above herbicides, with the exception of neburon, were applied to the two-year-old plants listed in Tables I and III. The plants were grown out of doors in washed silver sand in 10" clay pots sealed with bitumen and plugged with glass-wool; the pots were placed in bitumenized metal trays to catch any surplus liquid; this was re-applied to the surface of the pot. Chemicals were applied evenly over the surface of the sand by pipette and washed to the root zone by nutrient solutions (2). One pint of nutrient solution per pot was applied on alternate days and the sand was kept at field capacity with water. Other plants had single branches sprayed to run-off with solutions of the concentrations listed in Table III. The trees were kept under daily observation. The type of symptom produced was recorded and the time after application at which it appeared.

Seven varieties of strawberry were treated with simazine and neburon at the rates shown in Table II. The procedure was similar to that employed in the previous experiment except that 6" pots were used and the plants were grown in a glasshouse at 60°F under 16-hour-days. Five leaves per plant were treated in the foliar applications.

Results.

The effects of applying residual herbicides to fruit plants growing in sand are shown in Tables I and II.

TABLE I.

EFFECT OF FOUR RESIDUAL HERBICIDES ON FRUIT PLANTS GROWING IN SAND CULTURE.

Applied 22.6.60.

Fruit	Variety	Rootstock	Amiben 12 lb./ 100 gal./ acre	Simazine 6 lb./ 100 gal./ acre	Monuron 6 lb./ 100 gal./ acre	Diuron 6 lb./ 100 gal./ acre
Apple	Lane's Prince Albert	MII	0	0	K(2)	DD(3)
	Bramley		0	0	0	0
	Cox		0	0	0	0
Pear	Bristol Cross	Quince A	0	DD(7)	K(3)	DDD(4)
	Williams'		0	DD(7)	K(3)	DDD(4)
Plum	Victoria	Common Plum	DD(3)	0	K(3)	DD(4)
Raspberry	Malling Exploit	—	N.T.	DD(4)	K(2)	K(3)
Black currant	Cotswold Cross	—	0	DD(4)	K(2)	K(3)
Red currant	Red Lake	—	D(3)	DD(4)	K(2)	DDD(3)

TABLE II.

EFFECT OF NEBURON AND SIMAZINE ON STRAWBERRY PLANTS GROWN IN SAND.

Applied 30.9.60.

Variety	Simazine $\frac{1}{2}$ lb./100 gal./acre	Simazine 1 lb./100 gal./acre	Neburon 1 lb./100 gal./acre
Favourite	0	0	0
Huxley	D(5)	DD(5)	DD(6)
Prizewinner	DD(4)	DDD(3)	DD(4)
Redgauntlet	DD(3)	DDD(3)	DDD(4)
Royal Sovereign	D(8)	DD(8)	DDD(8)
Talisman	D(6)	DD(6)	DDD(6)
Vigour	DD(3)	DDD(3)	DD(4)

Damage : 0=none visible ; D=very slight ; DD=moderate ; DDD=severe ; K=killed ; N.T.=not treated. Figures in brackets represent number of weeks after treatment for symptoms to appear.

Symptoms.

Amiben caused browning and abscission of the older leaves of plum and interveinal chlorosis of red currant leaves.

Simazine symptoms first appeared on unfolded young leaves, but damage often developed later in older leaves. Pear and black currant developed marginal and interveinal blackening, while the leaves of red currant suffered marginal and interveinal browning. Raspberry leaves became

bronzed and scorched around their margins and strawberry developed marginal and interveinal chlorosis followed by necrosis.

Neburon symptoms on strawberry were similar to those produced by simazine.

Diuron produced symptoms similar to those caused by simazine on the same range of plants; in addition the apple Lane's Prince Albert and the plum Victoria developed leaf bronzing. In all cases the damage appeared more quickly and was more severe than that produced by simazine.

Monuron affected the same fruit plants as diuron, but produced similar symptoms more rapidly and caused more damage.

The effects of applying herbicides to the foliage of fruit plants are shown in Table III.

TABLE III.
EFFECT OF FOUR RESIDUAL HERBICIDES ON THE FOLIAGE OF FRUIT PLANTS.
Applied 22.6.60

Fruit	Variety	Rootstock	Amiben 12 lb./ 100 gal./ acre	Simazine 6 lb./ 100 gal./ acre	Monuron 6 lb./ 100 gal./ acre	Diuron 6 lb./ 100 gal./ acre
Apple	Lane's Prince Albert	MII	D(2)	0	DD(2)	D(3)
	Bramley		D(2)	0	DD(2)	D(3)
	Cox		D(2)	0	D(2)	D(3)
Pear	Bristol Cross	Quince A	D(2)	0	D(2)	D(2)
	Williams'		D(2)	0	D(2)	D(2)
Plum	Victoria	Common Plum	D(3)	0	D(2)	D(3)
Raspberry	Malling Exploit	—	N.T.	0	DD(2)	DD(2)
Black currant	Cotswold Cross	—	0	0	DD(2)	DD(2)
Red currant	Red Lake	—	0	0	DD(2)	D(2)

Damage: 0=none visible; D=very slight; DD=moderate; DDD=severe; N.T.=not treated. Figures in brackets represent number of weeks after treatment for symptoms to appear.

No symptoms appeared following the application of neburon and simazine to the foliage of the seven varieties of strawberry given in Table II.

Symptoms.

Amiben caused interveinal chlorosis on the treated leaves of apple while treated pear leaves developed black lesions.

Simazine: no symptoms.

Neburon (applied only to strawberries): no symptoms.

Diuron caused interveinal chlorosis on treated leaves of apple, pear and plum and leaf bronzing on raspberry and black currant. Treated red currant leaves became completely pale brown.

Monuron symptoms were much the same as those produced by diuron. Some untreated leaves of red currants and black currants developed similar symptoms.

Discussion.

Of the herbicides tested in these experiments, the least damaging to fruit plants was amiben, but unfortunately this chemical has proved relatively ineffective for weed control under Long Ashton conditions.

When applied to the roots the substituted urea herbicides caused severe damage, resulting in the death of many of the fruit plants tested. The only exceptions found were the apple varieties Bramley's Seedling and Cox's Orange Pippin and the strawberry variety Favourite, which appear to possess a considerable degree of tolerance to these herbicides. In other fruits damage from monuron manifested itself more quickly than that from diuron, a difference which may be associated with the much greater water solubility of monuron (page 60). Likewise the fact that simazine, at 6 lb./acre, in general caused less severe and less immediate damage than did the substituted ureas at the same rate of application, may also be partly correlated with its low solubility. All three of the apple varieties tested, and Victoria plum and Favourite strawberry, apparently possessed innate tolerance to this herbicide; all other strawberry varieties were damaged by rates as low as $\frac{1}{2}$ lb./acre. The symptoms of simazine damage were essentially similar to those caused by the urea herbicides; this might perhaps be expected in view of their similar mode of action (3,4).

Simazine was also outstanding as being the only herbicide tested on both tree and bush fruits which caused no damage when applied to the foliage. Combined with the relatively high tolerance shown by fruit plants to root application, this would seem to make the margin of safety for this compound greater than that of the substituted ureas when used for weed control in fruit crops. Just how great the margin of safety is can only be assessed by field trials extending over a number of seasons. The results here presented suggest, however, that simazine at rates up to 6 lb./acre can safely be applied to apples and plums, which are deep-rooted plants with a high degree of tolerance. The danger of damage to black currants, raspberries and strawberries, which are relatively shallow rooted and have less tolerance, would appear to be much greater.

Acknowledgments.

I am indebted to Dr. L. C. Luckwill for his help and interest in this work, to Amchem Products Inc. for supplying amiben, to the Du Pont Company for supplying diuron, monuron and neburon, and to Fison's Pest Control for supplying simazine.

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THE USE OF RADIOACTIVE PHOSPHORUS FOR ASSESSING THE GROWTH OF POLLEN TUBES IN APPLE PISTILS.

By C. P. LLOYD-JONES and R. R. WILLIAMS.

For the investigation of pollen tube incompatibility in apple and pear, a technique was required for assessing the growth of pollen tubes in the flowers. Conventional methods of counting the tubes after staining failed to give good results, owing to the very large number and matted condition of the pollen tubes in the styles and ovaries of successfully pollinated flowers—often over 500 tubes per flower. Experiments were therefore carried out in which pollen was labelled with radioactive phosphorus and the pollen tube growth followed by radio-assay of the pollinated flowers.

Pollen was labelled by placing flowering spurs in a solution containing 10 p.p.m. phosphorus in the form of sodium dihydrogen phosphate with a specific activity of 1 mc. $^{32}\text{P}/2$ mg. P. The spurs were placed in the solution when the blossoms were in the early "balloon" stage, and were left until the petals opened and the anthers were about to dehisce, usually for a period of two to four days. The flowers were then picked and, after removal of the petals, were allowed to dry in a warm room overnight. The pollen was then rubbed off the anthers for use in pollination, for radio-assay and for the germination to be checked in a hanging drop culture using a 10% sucrose solution. For radio-assay, tissues were digested in nitric acid, made to 25 ml. with water and the digest was counted in a liquid M6 type GM tube.

The uptake of ^{32}P by the pollen was usually much greater in apple than in pear. Thus, the pollen from a single flower of the cider variety Foxwhelp gave 5.2 counts/sec. whilst the perry pear Yellow Huffcap gave only 0.8 counts/sec. With other varieties of apple the uptake of ^{32}P was very variable, this variation apparently being correlated with the transpiration potential of the flower truss. Varieties with several expanded leaves on the flowering spurs at blossom-time readily took up the ^{32}P , and high counts were obtained from the pollen. Likewise, large expanded petals stimulated uptake of ^{32}P . Varieties which carried little leaf area at flowering time, and had small petals, never took up sufficient ^{32}P to give adequately labelled pollen. The difference in uptake is illustrated by the following mean counts/sec. per flower obtained with three varieties of cider apple:

<i>Brown Snout</i> : many leaves and big flowers on spur	151
<i>Harry Master's Jersey</i> : some leaves and medium sized flowers	108
<i>Royal Jersey</i> : no leaves, small flowers	= 43

In order to follow the growth of pollen tubes in the pistil, flower trusses of the variety to be pollinated were emasculated at anthesis, labelled pollen was applied to the stigma with a camel hair brush, and the flowers were sampled at intervals. After picking, the petals and sepals were removed and the receptacle was thoroughly scraped to remove any surface contamination with radioactive pollen. The gynaecium was then cut into the following portions: stigmas, free styles, stylar column, and receptacle with ovary, using a separate razor blade for detaching each portion. Fifteen to twenty flowers per sampling were found to give convenient counts.

Samples, other than the stigmas, were washed in detergent and acid-digested for counting. Table I gives typical results obtained after a cross pollination with highly compatible pollen.

TABLE I.
PERCENTAGE DISTRIBUTION OF ^{32}P IN FLOWERS OF PERTHYRE AFTER POLLINATION WITH
LABELLED HARRY MASTER'S JERSEY POLLEN.

<i>Interval from pollination to sampling (hr.)</i>	<i>Stigmas</i>	<i>Free styles</i>	<i>Stylar column</i>	<i>Ovary</i>
0	100	0	0	0
24	76	24	0	0
24	83	14	3	0
96	42	20	20	18

It is concluded that the method, although tedious, could be valuable in comparing the relative efficiencies of several pollens, provided that the pollen-producing varieties had leaves or petals large enough to ensure adequate uptake of ^{32}P . It would therefore not be suitable for perry pears or other fruits which produce little leaf before petal fall.

A SIMPLE PHOTO-ELECTRIC DEVICE FOR MEASURING THE AREAS OF DETACHED LEAVES.

By DOROTHY I. CONIBEAR and C. G. L. FURMIDGE.

Measurement of the light extinction produced when leaves are placed in front of a light source can provide a very rapid and reasonably accurate assessment of leaf area. Several methods based on this principle have been published (1-3); the advantages of the method here described lie in the simplicity of the equipment relative to its high sensitivity.

Apparatus.

The requirements of a light extinction method are listed by Maggs (3): they include (a) a constant light source, (b) uniform illumination of the subject, and (c) a known and sensitive relation between the reading obtained and the leaf area. The first and second of these requirements are met by using a cold cathode grid C.C. (Fig. 1) as a light source. This is run from a constant voltage transformer to avoid fluctuations in light intensity due to mains voltage variation; it provides a fairly uniform illumination over an area 18×18 cm.

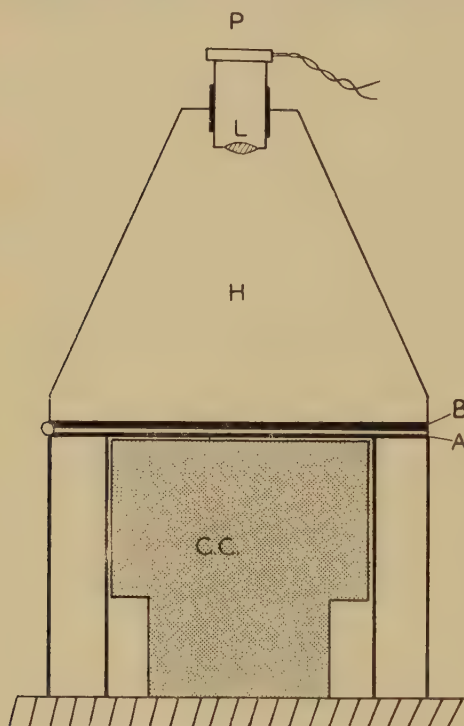


Fig. 1. Lay-out of equipment.

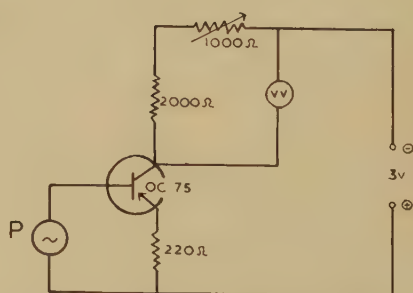


Fig. 2. Circuit diagram for photocell.
P=G.E.C. Rectifier Type 1 Photocell.
V.V.=Pye Universal pH and Voltmeter.
OC 75=Mullard Transistor.

Mounted directly above the light source is a sheet of glass A, which is painted black except for a clear window in the centre to accommodate the

leaves. The area of this window may be varied according to the number and size of leaves to be measured ; for greatest accuracy it should be kept as small as possible. For the assessment of laurel, apple and bean leaves, two window sizes have been used : 60 cm.^2 for measuring single leaves and 200 cm.^2 for measuring groups of three or four leaves. One side of this sheet of glass is hinged to a second glass sheet B which is mounted in the base of a pyramidal hood H ; the inside of this hood is painted matt black. The leaves are placed between A and B, thin spacers being fitted so that the leaves are pressed flat but are not crushed. An $f 2.8$ camera lens L, and a selenium barrier photocell P, are fitted in the top of the hood so that the window in A is focused on the face of the photocell. The signal from the photocell is amplified as shown in the circuit diagram, Fig. 2, and measured on the valve voltmeter V.V. The use of a valve voltmeter is not essential ; any accurate millivoltmeter with a high input resistance would be suitable.

Operation and Accuracy.

With the window in A left clear, the variable resistor is adjusted until a suitable "zero" reading is obtained on the voltmeter. The magnitude of this reading depends on the window area employed ; satisfactory results have been obtained using a reading of 500 mv. with the 60 cm.^2 window and 1,000 mv. with

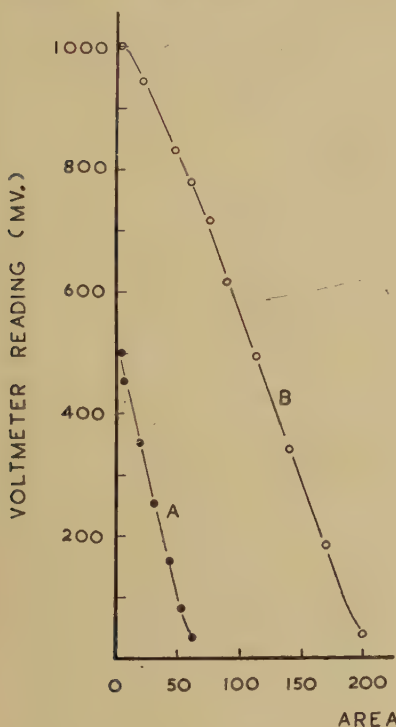


Fig. 3. Calibration curves using metal squares.
Curve A = 60 cm.^2 window.
Curve B = 200 cm.^2 window.

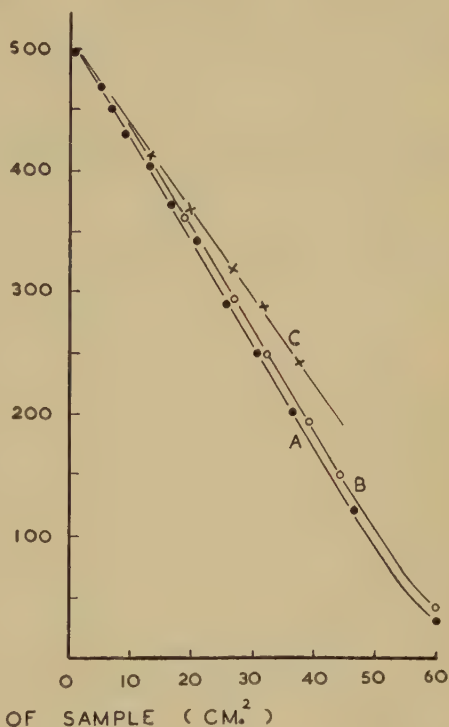


Fig. 4. Calibration curves for the 60 cm.^2 window.
Curve A = metal squares.
Curve B = sections of banana leaf.
Curve C = laurel leaves.

the 200 cm.² window. The leaves are placed in the window and the new reading noted from the voltmeter ; the zero reading should be checked occasionally between area measurements. The leaf area is then read from the voltage/area calibration curve.

The photocell response is approximately linear over most of the area range, as shown by curves A and B, Fig. 3a ; these curves were obtained using standard metal squares. Leaves transmit a small amount of light and allowance has to be made for this when measuring leaf area ; for this reason it is desirable to calibrate the instrument by using the type of leaf that is to be measured. Calibration curves for laurel leaves and sections of banana leaves using the 60 cm² window are shown in curves B. and C, Fig. 3b.

The reproducibility of results was checked by making ten successive measurements on a single laurel leaf, using the 60 cm² window, and also ten measurements on a group of three laurel leaves, using the 200 cm² window. The first set of results gave a mean area of 19.61 cm² with a coefficient of variation of 1.14% ; the second set gave a mean area of 66.9 cm² with a coefficient of variation of 0.85%.

Acknowledgments.

The authors are indebted to Dr. E. J. Skerrett for advice on the amplifier and measuring circuit.

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MANURIAL EXPERIMENTS WITH FRUIT : IV. STRAWBERRY UNDER CLOCHES.

By C. BOULD.

Previous papers (1, 2) have dealt with the effect of long-term fertilizer and F.Y.M. treatments on soil fertility, crop yield and leaf nutrient status of strawberry. These long-term experiments showed the importance of potassic fertilizers, the relatively low requirement for nitrogenous fertilizers and the absence of response to phosphate. A more recent paper (3) dealt with the short-term effects of NPK fertilizers, in factorial combination, on the growth, crop yield and leaf nutrient status of strawberry, var. Royal Sovereign. It was shown that virus-tested plants, provided they have been well supplied with nutrients in the runner bed, have a relatively low fertilizer requirement in the field. Furthermore, the results indicated that, if the concentrations of nitrogen, phosphorus and potassium in fully expanded physiologically active leaves, sampled at the fruit-ripening stage, are above certain values (sufficiency values) then the plants are unlikely to respond to fertilizer applications in the following spring.

The present paper deals with the effect of NPK fertilizers on the growth, crop yield and leaf nutrient status of strawberry under cloches at the Rosewarne Experimental Horticulture Station, and illustrates the close relationship that exists between plant nutrient status, leaf analysis at the fruiting stage, and the response to fertilizers.

Experimental.

Soil.

Rosewarne Series. This is a brown earth type, freely drained, depth varying from 12 to 30". The top soil is dark brown, passing through a reddish brown loam to a reddish-brown clay loam subsoil. Chemical analyses of the soil, prior to fertilizer treatments, are given in Table I.

Layout and Manurial Treatments.

The total experimental area is approximately 0.26 acres and is divided into 3 blocks each containing 27 plots. A plot consists of 4 rows each of 12 plants, 12" between rows and 15" between plants in the row. There is a clear 3' 1½" between plots in the N-S direction (within rows) and 5' between the outside rows of adjoining plots in the E-W direction. The plants are covered by double rows of barn type cloches running N-S.

There are 27 treatments, in triplicate, comprising all combinations of N, P and K. In the first experiment the following rates per acre were applied over the whole plots prior to planting, starting on September 20, 1954.

$N_0; N_1; N_2 = 0; 1; 2$ cwt. ammonium sulphate.

$P_0; P_1; P_2 = 0; 2; 4$ cwt. superphosphate (approx. 18% P_2O_5).

$K_0; K_1; K_2 = 0; 1\frac{1}{2}; 3$ cwt. potassium sulphate.

In the second experiment, made in 1959-60, the potassium sulphate was reduced to 1 and 2 cwt. per acre for K_1 and K_2 respectively.

Between the two strawberry experiments violets, anemones and cover crops occupied the site. The fertilizer rates for violets were the same as for the first strawberry experiment, but for anemones the rates were changed to :—

$N_0; N_1; N_2 = 0; 2; 4$ cwt. ammonium sulphate.
 $P_0; P_1; P_2 = 0; 3; 6$ cwt. superphosphate.
 $K_0; K_1; K_2 = 0; 3; 6$ cwt. potassium sulphate.

The rotation of crops was as follows :—

1953/54 Ley; 1954/55 Strawberry, var. Royal Sovereign;
 1955/56 Anemones; 1956/57 Violets; 1957/58 Ley-Rye grass;
 1958/59 Cereal—Oats and grass; 1959/60 Strawberry var. Cambridge
 Favourite.

Planting Dates and Management.

In the first experiment virus-tested runners, variety Royal Sovereign, were planted between September 23 and 27, 1954. Normal commercial cultivations and spray treatments were given throughout. Cloches were placed over the plants on February 14, 1955. The first fruits were picked on May 16 and picking was completed on June 24.

In the second experiment virus-tested runners of the variety Cambridge Favourite were planted between September 28 and 30, 1959. Irrigation was applied for 2 hr. directly after planting. The plants were covered with cloches on February 2, 1960. At this time the no-phosphate plots were practically weed-free whilst plots receiving phosphate carried a normal weed cover; this indicated the very low phosphorus status of the no-phosphate plots. The first fruits were picked on May 6 and picking was completed on June 7.

Leaf Samples.

Leaf samples, consisting of one fully expanded leaf of intermediate age, with the minimum amount of petiole attached, were taken from every other plant at early fruit ripening. The dates were :—1st experiment=May 24, 1955; 2nd experiment=May 13, 1960. The samples were dried at 100°C, crushed, sub-sampled and analysed for total nitrogen, phosphorus and potassium (4).

Experimental Records.

Records have been made of plant vigour, plant losses, incidence of disease and pests, yield and grade of fruit, and leaf nutrient status.

Results.

Soil Analysis.

Analytical data for top- and sub-soil are given in Table I. The soil is slightly acid, low in exchangeable potassium and very deficient in "available" phosphorus. The organic matter status is reasonably normal and exchangeable magnesium is satisfactory for the level of potassium present. Table II shows the effect of some of the treatments (1954-59) on exchangeable potassium and "available" phosphorus. The mean exchangeable potassium was almost trebled by the higher rate of potassic fertilizer, whilst the mean "available" phosphate was nearly doubled by the highest phosphate dressings; the new phosphate value was still low. Ammonium sulphate had no acidifying effect on soil pH.

TABLE I.

SOIL ANALYSES PRIOR TO MANURIAL TREATMENTS, 1954.
(As % air-dry soil).

Depth	pH Soil:water; 1:2.5	Organic carbon	Exchangeable		N/2 acetic sol. P	Total P
			K	Mg		
0—8"	5.9	3.96	0.0062	0.0103	0.00035	0.134
8—16"	6.3	2.67	0.0052	0.0093	0.00012	—

TABLE II.

SOIL ANALYSES AFTER MANURIAL TREATMENTS, 1960.
(Means of 3 plots as % air-dry soil, 0—8")

Treatment	pH	Exchangeable K	N/2 acetic sol. P
N ₀ P ₀ K ₀ (control)	6.2	0.0072	0.00054
N ₂ P ₀ K ₀	6.3	0.0058	0.00046
N ₀ P ₂ K ₀	6.7	0.0032	0.00077
N ₂ P ₂ K ₀	6.5	0.0056	0.00088
N ₀ P ₀ K ₂	6.0	0.0172	0.00038

Plant Vigour and Symptoms.

Royal Sovereign. There were no major differences in growth due to treatments. Growth was fairly vigorous except in severely mildewed plants. There were no deficiency symptoms.

Cambridge Favourite. An assessment of vigour was made on May 13, at the time of leaf sampling. Plants were given markings for vigour from 1 (very small) to 5 (very vigorous). The results are summarized below as totals from 27 plots.

Plant Vigour

N ₀ =79	P ₀ =77	K ₀ =74
N ₁ =81	P ₁ =83	K ₁ =84
N ₂ =88	P ₂ =86	K ₂ =89

There was a small general response to all three fertilizers, the greatest response being to potassic fertilizer. There were no deficiency symptoms.

Yield of Fruit.

Royal Sovereign. The results for total crop only are given in Table III. There was a small but positive effect due to nitrogen (N₂-N₁ but not N₂-N₀) and phosphate, both at the 5% level of probability. There was no significant effect due to potassium, but a significant NK interaction (5% level).

Adjusted NK Means (oz./plot 48 plants)

	N ₀	N ₁	N ₂
K ₀	89.3	78.3	82.8
K ₁	78.6	79.6	80.9
K ₂	74.5	68.4	89.3

The highest yields were given by N₀K₀ and N₂K₂; all other combinations gave lower yields.

There was a significant block effect (1% level); yields from Block II were much lower than those from Blocks I and III.

Cambridge Favourite. Yields of total and Grade I fruit (less than 50 fruits/lb.) are shown in Table III. There were no significant differences due to fertilizers. Yields from Block I were much less than those from Blocks II and III.

Plant and Leaf Analyses.

Royal Sovereign. Prior to planting, 20 runners were taken for chemical analysis. They were divided into leaves, roots and crowns and analysed for total nitrogen, phosphorus and potassium. The results are given below, as percentages of dry matter.

Analysis of Runners, 19.7.54

	<u>Total N</u>	<u>Total P</u>	<u>Total K</u>
Leaves	1.94	0.249	1.89
Roots	0.86	0.147	1.89
Crowns	1.56	0.315	2.65

It will be seen that, compared with roots and leaves, the crowns had a fairly high concentration of phosphorus and potassium.

Leaf analyses are given in Table IV. There was no effect on leaf-N or leaf-P due to fertilizer treatment. The response to potassic fertilizer was significant at the 0.1% level of probability. Phosphatic fertilizer had a highly significant (0.1% level) depressing effect on leaf-N.

Percentage Nitrogen in Leaves

P₀=2.74 ; P₁=2.71 ; P₂=2.63

Cambridge Favourite. Leaf analyses are given in Table IV. Nitrogenous fertilizer had no significant effect on leaf-N; phosphatic fertilizer had a significant positive effect (5% level) on leaf-P, and potassic fertilizer had a highly significant effect (0.1% level) on leaf-K. Phosphatic fertilizer had a significant (5% level) depressing effect on leaf-N as shown below :—

Percentage Nitrogen in Leaves

P₀=3.24 ; P₁=3.19 ; P₂=3.09

TABLE III.
YIELDS OF FRUIT PER PLOT.
(Means for 27 plots in oz./plot 48 plants).

Variety	Nitrogen			Sig. Diff.	Phosphorus			Sig. Diff.	Potassium			Sig. Diff.
	N ₀	N ₁	N ₂		P ₀	P ₁	P ₂		K ₀	K ₁	K ₂	
<i>Royal Sovereign</i> , 1955 Total Crop*	80.8	75.5	84.3	6.9 (5%)	76.9	77.9	85.7	6.8 (5%)	83.4	79.7	77.4	N.S.
<i>Cambridge Favourite</i> , 1960 Total Crop Grade I	113.4 51.5	109.4 47.5	119.6 53.5	N.S. N.S.	110.5 50.5	114.0 49.6	117.8 52.4	N.S. N.S.	117.9 54.4	111.0 49.6	113.5 48.5	N.S. N.S.

*Adjusted for plant number and severely mildewed plants. N.S.=not significant.

TABLE IV.
LEAF COMPOSITION AT THE FRUITING STAGE.
(As % dry matter; means of 27 plots).

	Nitrogen			Sig. Diff.	Phosphorus			Sig. Diff.	Potassium			Sig. Diff.
	N ₀	N ₁	N ₂		P ₀	P ₁	P ₂		K ₀	K ₁	K ₂	
<i>Royal Sovereign</i> , 1955	2.70	2.68	2.70	N.S.	0.253	0.256	0.249	N.S.	1.14	1.45	1.60	0.11 (0.1%)
<i>Cambridge Favourite</i> , 1960	3.15	3.18	3.20	N.S.	0.300	0.308	0.304	0.007 (5%)	1.51	2.09	2.42	0.19 (0.1%)

N.S.=not significant.

Discussion.

It is clear from the results of these two experiments that fertilizer treatment has very little effect on the yield of strawberries grown for one season under cloches in a soil of low fertility, provided that well-nourished, virus-tested runners are used and that the physical condition of the soil is satisfactory. Analysis of the runners used in the first experiment indicated ample supplies of phosphorus and potassium in the crowns. These reserves must have been sufficient for the runners to make satisfactory root and shoot growth on a soil very low in "available" phosphate and exchangeable potassium. Chemical analysis of leaf samples of Royal Sovereign, taken at the fruiting stage, showed that the levels of nitrogen and phosphorus were satisfactory and unaffected by fertilizer treatment. Similar results were obtained for Cambridge Favourite, although the levels were generally higher. It has been observed from other leaf analyses (unpublished) that leaf-nitrogen and leaf-phosphorus levels are lower in Royal Sovereign than in some other commercial varieties, e.g. Vigour, Talisman and Climax, when grown on the same site.

In both experiments there was a significant effect of potassic fertilizers on the concentration of leaf-potassium, but this was not associated with enhanced yields. The leaf-K value of 1.14% in dry matter of the control (no potassium) Royal Sovereign plants was below the optimum value (about 1.6% K) and would no doubt have affected the yield from these plants had they been cropped for a second season. It would appear, however, that a good supply of potassium in the crowns of runners at the time of planting (September) will enable the plants to carry a normal crop for one season on soils low in exchangeable potassium.

No data are available for the chemical composition of the Cambridge Favourite runners, but it will be seen from Table IV that the composition of the leaves, taken in the following season from control plots ($N_0P_0K_0$), at the time of fruit ripening, was satisfactory for total N, P and K. This would explain the lack of response to fertilizers.

The conclusion to be drawn from these two experiments is that the nutrient status of the runner is more important than the fertility of the cropping site for strawberries cropped for one season only under cloches. It is hoped to investigate this problem further, and to determine sufficiency levels of nutrients for runner crowns in the same manner as for leaves.

Acknowledgments.

I am greatly indebted to Mr. F. W. Shepherd, Director of the Rosewarne Experimental Horticulture Station, for his continued interest and help in these experiments, and to Mr. E. G. Ing who was responsible for general management and records. Thanks are also expressed to the Statistics Section, East Malling Research Station, for the design and layout, to Mr. G. M. Clarke of this Station for the analysis of the results, and to Mr. E. G. Bradfield and Mr. W. K. Hancock for the chemical analyses.

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SPRAY CHEMICALS AND THE FRUITGROWER.

By J. T. MARTIN.

Immediately after the last war, new synthetic insecticides such as DDT, gamma-BHC, TEPP and schradan were competing strongly with nicotine, tar oil and other survivors from the pre-war period. The age of the synthetic chemical had begun, and many more materials have since been developed. As new remedies emerge, the grower tends to find difficulty in differentiating between them and in deciding upon those most suitable for his purposes. Some of the materials in use are toxic to man and animals and others persist for long periods on foliage and fruits; the grower therefore also wishes to know whether hazards are likely to arise to persons applying the washes, to consumers of treated crops or to wildlife. These difficulties have been recognized and steps have been taken to overcome them. The purpose of this article, prompted by queries raised in discussions with growers, is to review the present position of fruit sprays and to draw attention to the recommendations made for their effective and safe use.

Nomenclature.

Unlike the relatively simple pre-war materials, the new synthetics are often complex organic compounds with chemical names incomprehensible to the layman. The need arose for short non-proprietary names by which the active ingredients of commercial products could be known to the users. The coining of such names was undertaken by the British Standards Institution in 1949 and common names have now been adopted for many insecticides, fungicides, herbicides and rodenticides (1). The Institution collaborates with similar organizations overseas to secure international acceptance of the names proposed.

For adoption, a common name must be easily pronounced and readily remembered, must not clash with another registered name, and desirably should give a clue to the chemical nature of the compound to which it refers. As an example, the proprietary remedy Melprex contains as its active ingredient *n*-dodecylguanidine acetate to which the common name dodine acetate (from the italicized syllables) has been given.

The use of common names has greatly helped the research worker in reports on chemicals, but the grower may be confused unless the relationship between common and proprietary names is clear. Proprietary and common names are often wrongly used synonymously: the proprietary name refers to the commercial formulation and the common name to the active chemical in it. A number of products bearing different proprietary names yet all containing the same active ingredient may thus be available. Confusion also arises from undue delay in the adoption of common names. A product containing a new chemical is marketed and its proprietary name becomes established; later a common name is given to its active principle and this name also begins to appear in the technical literature. A grower, having used a proprietary material, may see reference to the new name of its active component and think it another remedy. The difficulties are overcome to some extent by the established practice, followed in this review, of using a capital initial letter for a proprietary

and a small initial letter for a common name, and by the manufacturer giving on his label the common name of the active ingredient in addition to the proprietary name of the product.

The Spray Chemicals.

Insecticides and Acaricides.

Few of the pre-war materials are now used to any great extent. Soon after their introduction, the chlorinated hydrocarbon compounds DDT, gamma-BHC, endrin, chlorbenside, chlorfenson and chlorobenzilate, and the organo-phosphorus compounds parathion, schradan, demeton, dimefox, TEPP and sulfotep, became established for the control of a wide range of fruit insects and/or mites. The long-established winter washes gave way to spring washes, e.g. of DDT and gamma-BHC, and a new method of insect control was introduced with organo-phosphorus compounds that acted systemically within the plant.

Organo-phosphorus substances have predominated among the remedies recently introduced. The chemical natures and properties of the compounds have been reviewed elsewhere (2); those already used by or becoming familiar to fruit growers are shown in Table I.

TABLE I.
ORGANO-PHOSPHORUS INSECTICIDES AND ACARICIDES.

<i>Common name</i>	<i>Chemical name</i>	<i>Proprietary formulation</i>
azinphos-methyl	<i>S</i> -(3,4-dihydro-4-oxobenzo[d]-[1,2,3]-triazin-3-ylmethyl) dimethyl phosphorothiolothionate	Gusathion
demeton-methyl	<i>S</i> -[2-(ethylthio)ethyl] dimethyl phosphorothiolate (and thionate)	Meta-systox
diazinon	diethyl 6-methyl-2-isopropyl-4-pyrimidinyl phosphorothionate	Basudin
dimethoate	<i>S</i> -(<i>N</i> -methylcarbamoylmethyl) dimethyl phosphorothiolothionate	Rogor
ethion	tetraethyl <i>SS'</i> -methylene bis(phosphorothiolothionate)	Nialate
malathion	<i>S</i> -[1,2-di(ethoxycarbonyl)ethyl] dimethyl phosphorothiolothionate	Malathion
mecarbam	<i>S</i> -(<i>N</i> -ethoxycarbonyl- <i>N</i> -methylcarbamoylmethyl) diethyl phosphorothiolothionate	Murfotox
mevinphos	2-methoxycarbonyl-1-methylvinyl dimethyl phosphate	Phosdrin
morphothion	<i>S</i> -(morpholinocarbonylmethyl) dimethyl phosphorothiolothionate	Ekatin M
phenkapton	<i>S</i> -(2,5-dichlorophenylthiomethyl) diethyl phosphorothiolothionate	Phenkaptone
phosphamidon	2-chloro-2-diethylcarbamoyl-1-methylvinyl dimethyl phosphate	Dimecron

No attempt has been made to list all the proprietary formulations. The products are marketed for the control of aphids, red spider mites and other pests such as codling moth, tortrix and sawfly. Demeton-methyl, dimethoate, mevinphos, morphothion and phosphamidon are systemic in action.

Other insecticides and acaricides that are not members of the phosphorus group are shown in Table II.

TABLE II.
NON-PHOSPHORUS INSECTICIDES AND ACARICIDES.

<i>Common name</i>	<i>Chemical name</i>	<i>Proprietary formulation</i>
—	1,1,1-trichloro-2,2-bis(<i>p</i> -chlorophenyl) ethanol	Kelthane
fluoroacetamide	fluoroacetamide	Megatox
—	1,1-dichloro-2,2-bis(<i>p</i> -chlorophenyl) ethane	Rothane
—	1-naphthyl- <i>N</i> -methylcarbamate	Sevin
tetradifon	2,4,5,4'-tetrachlorodiphenyl sulphone	Tedion
—	6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepine 3-oxide	Thiodan

Sevin is of interest for the control of pests of top fruits, and Thiodan has been proposed for use against the black currant gall mite. Fluoroacetamide is a systemic compound that may be used on strawberries. Rothane is an insecticide similar to DDT; tetradifon and Kelthane are acaricides.

Fungicides.

Captan, dithiocarbamates and aryl (organo) mercury compounds are now well established, and few new fungicides have been developed. The newer materials and those in which interest is shown are given in Table III.

Dinocap is used against powdery mildews, and dodine acetate, Glyodin and etem (see p. 82) are under trial for scab control. Phaltan is closely related chemically to captan.

Herbicides.

Remarkable advances have been made in herbicides; some forty common names of new compounds are listed in the 1960 Weed Control Handbook (3). Research is actively in progress at many centres to establish the tolerance of fruiting plants to the new materials (see p. 59). Recommendations for the use of compounds such as simazine (2-chloro-4,6-bisethylamino-1,3,5-triazine) and dalapon-sodium (sodium 2,2-dichloropropionate) in fruit plantations are given in the Handbook.

TABLE III.
FUNGICIDES.

<i>Common name</i>	<i>Chemical name</i>	<i>Proprietary formulation</i>
dinocap	2-(1-methyl- <i>n</i> -heptyl)-4,6-dinitrophenyl crotonate	Karathane
dodine acetate	<i>n</i> -dodecylguanidine acetate	Melprex
etem	hexahydro-2,7-dithio-1,3,6-thiadiazepine	UCP 21
—	2-heptadecyl-2-imidazoline acetate	Glyodin
—	<i>N</i> -trichloromethylthiophthalimide	Phaltan

Precautions in the Use of Spray Chemicals.

The potential hazards of spray chemicals to operators, consumers and wildlife were examined from 1950 onwards by interdepartmental committees. Following their reports (4) statutory requirements were imposed and voluntary measures were recommended to ensure the safe use of the chemicals.

TABLE IV.
FRUIT SPRAYS AND OPERATOR PROTECTION.

<i>Specified compounds</i>		<i>Operation*</i>	<i>Protective clothing</i>
Part I	demeton, dimefox	(a)	Rubber gloves, rubber boots, respirator and either an overall and rubber apron <i>or</i> mackintosh.
		(b)	Rubber coat, rubber gloves, rubber boots, sou'wester and respirator.
		(c)	Rubber boots, face-shield and either an overall and rubber apron <i>or</i> mackintosh.
Part II	endrin, fluoroacetamide, mevinphos, parathion, schradan, sulfotep, TEPP, Thiodan	(a)	Rubber gloves, rubber boots, face-shield and either an overall and rubber apron <i>or</i> mackintosh.
		(b)	Rubber coat, rubber gloves, rubber boots, sou'wester and face-shield.
		(c)	Rubber boots, face-shield and either an overall and rubber apron <i>or</i> mackintosh.
Part III	azinphos-methyl, demeton-methyl, ethion, mecarbam, phenkapton, phosphamidon	(a)	Face-shield and rubber gloves.
		(b)	None
		(c)	None

**Operation* (a) opening a container, or diluting, mixing or transferring from one container to another; (b) spraying bushes or trees; (c) washing or cleansing spraying machine.

Operators.

After the first report, the Agriculture (Poisonous Substances) Regulations were introduced to protect employees from poisoning when applying the more dangerous compounds. A summary and explanation of the Regulations are given in an official leaflet (5) which should be seen by all concerned with spraying operations. The leaflet defines the obligations on employers and employees, lists the protective clothing which must be worn when specified poisons are used for certain purposes, discusses the precautions to be taken against spray drift, the poisoning of livestock and wildlife (including beneficial insects and fish) and makes recommendations for the safe disposal of containers.

The potentially dangerous chemicals are classified as Parts I, II, III and IV substances, the most toxic being Part I substances. Table IV shows the specified compounds used on fruit crops and indicates the protective clothing required when they are used out-of-doors.

Part IV substances are the organo-mercury aerosols; special precautions are needed when any specified compound is used under glass. Demeton is not recommended for fruit spraying and dimefox is used only on strawberries.

Sevin, diazinon, dodine acetate, chlorobenzilate, lead arsenate, malathion, dimethoate, morphothion, organo-mercury foliage sprays, simazine and tetradifon have been considered but are not included under the Regulations controlling operator use. The fungicides and many of the herbicides are considered to be sufficiently non-toxic to be safe to persons applying them. It is, however, stressed that when using any spray chemicals sensible precautions should be taken. These include avoiding contact of the concentrated material with the skin, washing the skin and eyes immediately and thoroughly if contaminated, avoiding undue wetting and the continued use of soaked clothing, and washing exposed parts of the body after use and before eating.

Consumers.

After the second report, an Advisory Committee to Government Departments was set up to deal with possible hazards to the consumers of treated crops. The Committee, assisted by a Scientific Sub-Committee, assesses information from the manufacturers and research stations on the contamination of food-stuffs with potentially dangerous chemicals and makes recommendations for their safe use. Maximum rates of application that may be used are stated, and the minimum periods that should be allowed to elapse between the last pre-harvest treatments and harvesting are stipulated (6). If the conditions laid down are observed, hazards to consumers are not likely to arise.

The recommendations for fruit sprays used out-of-doors are summarized in Table V. Where the maximum number and rate of applications are not shown, limits have not been imposed. Unless otherwise stated, the rates of application are given in terms of the active ingredients.

Where rates are expressed as quantities per 100 gallons, these are intended for large volume application; if applied in medium or small volume, the amounts of active ingredients used per acre should not exceed those which would have been applied in large volume.

Some materials now in use have still to be considered. To safeguard the future position the Ministry of Agriculture Fisheries and Food has introduced a notification scheme (7) by which manufacturers intending to market new or

revised formulations with potential hazards voluntarily disclose toxicity and other relevant information to obtain "clearance" or permit precautionary measures to be introduced.

TABLE V.
FRUIT SPRAYS AND CONSUMER PROTECTION.

<i>Compound</i>	<i>Crop</i>	<i>Maximum number and rate of applications</i>	<i>Time of application : minimum period before harvest</i>
azinphos-methyl	apple	Five, each at $7\frac{1}{2}$ oz./100 gal.	3 weeks
	pear	Three, each at $7\frac{1}{2}$ oz./100 gal.	3 weeks
chlorobenzilate	any crop	—	3 weeks
demeton-methyl	apple	Three each at 16 fl. oz.*/100 gal.	One pre-, two post-blossom ; 3 weeks
	pear, plum	Three, each at 16 fl. oz.*/100 gal.	Third after harvest ; 3 weeks
	black currant	Two, each at 16 fl. oz.*/100 gal.	Second after harvest ; 3 weeks
	strawberry	Three, each at 16 fl. oz.*/100 gal.	One before flowering, two after harvest
diazinon	any crop	—	2 weeks
dimefox	strawberry	One, 2 lb./acre	Before flowering begins
dimethoate	any crop	—	1 week
dodine acetate	apple	—	2 weeks
endrin	apple	—	Not later than 1 week after 80% petal-fall
	pear, cherry	—	Not after white bud
	black currant	—	Not later than immediately before flowering
	black currant (for fruit)	Two, each not exceeding 0.04%	First before flowering, second as soon after flowering as possible
	black currant (for juice)		4 weeks
ethion	apple, pear	Two, each at 8 oz./100 gal.	
fluoroacetamide	strawberry	One, at 3 oz./acre	4 weeks
lead arsenate	apple	—	6 weeks
malathion	any crop	—	1 week
mecarbam	apple, pear	Four, each at 10 oz./100 gal.	2 weeks
mevinphos	apple, pear } plum cherry } strawberry }	4 oz./acre per application 2 oz./acre per application	3 days 3 days
aryl-mercury	apple, pear	—	Up to end of July ; 6 weeks
phenkapton	apple, pear	—	2 weeks
	black currant	—	4 weeks
phosphamidon	apple, pear	Five, each at 5 oz./100 gal.	3 weeks
schradan	apple, peach	One, at 12 fl. oz./100 gal.	Up to end of July ; 4 weeks
	strawberry	Two, total 32 fl. oz./acre	First before flowering, second after harvest
Sevin	apple, pear	—	1 week
Thiodan	black currant	Two, each at 8 fl. oz./100 gal.	At first open blossom stage and 3 weeks later ; 6 weeks

* Of 50 per cent formulation.

The Selection of Chemicals.

In 1942, a voluntary scheme was introduced by which manufacturers could obtain official approval of their products and of the claims made for pest and disease control. The scheme had the disadvantage that approval largely depended upon evidence of efficiency in commercial use, leading inevitably to considerable delay in the granting of approval. A new Agricultural Chemicals Approval Scheme (8), introduced in 1960, seeks to overcome this difficulty by considering new materials while in the developmental stage, so that approval may be given at the time of marketing. The first publication under the Scheme (8) should be of considerable interest to growers ; it lists approved materials, denotes their active ingredients, indicates the pests and diseases controlled, and gives warnings concerning damage to some crops and possible hazards to operators, consumers and wildlife. The approval of a product containing a toxic chemical cannot be given until its safety in use has been considered and precautions, if necessary, have been recommended. The precautions are then given on the label of the approved product.

Conclusions.

The Regulations, recommendations and the list of approved products are of the greatest value in ensuring the safety of operators, consumers and wildlife and in enabling the grower to select materials satisfactory for his purpose. The measures introduced should be widely known ; the publications listed under references (5) and (8) and information on the safe use of any specified poison (6) are obtainable free of charge on application to the Ministry of Agriculture, Fisheries and Food, 5-8 St. Andrews Place, London, N.W.1. So far as risks to operators are concerned, the position is encouraging ; a high proportion of the phosphorus compounds recently introduced show lower toxicities to man than the original members of the group. The protection of the consumer from possibly harmful residues of some fruit sprays largely depends upon the growers' knowledge of the recommendations made for safe use and their translation into practice. The co-operation of the manufacturers in the notification scheme has been of the greatest benefit in ensuring that the labels of potentially harmful materials carry suitable directions for use.

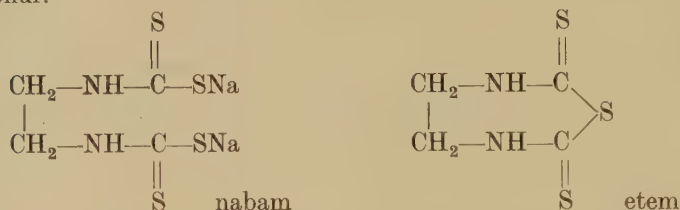
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SPRAY APPLICATION PROBLEMS: LXIII. **THE PERSISTENCE OF ETHYLENETHIURAM** **MONOSULPHIDE DEPOSITS.**

By J. T. MARTIN and R. J. W. BYRDE.

Ludwig and Thorn (1) have shown that the fungicide nabam* (disodium ethylenebisdithiocarbamate) breaks down under favourable conditions to a mixture of ethylenethiuram monosulphide (etem*), its polymer and elemental sulphur.



Ludwig, Thorn and Miller (2) ascribed the protective fungicidal action of nabam to the formation of etem, which they detected on the foliage of nabam-sprayed plants. Later, Ludwig, Thorn and Unwin (3) demonstrated the conversion of etem to an isothiocyanate (postulated by Sijpesteijn and van der Kerk (4) as the active principle) and concluded that the mechanism of fungicidal action of the zinc and manganese salts (zineb* and maneb*) was the same as that of the sodium salt. The possibility arose of using etem itself as a fungicide, and a few years ago a proprietary dispersible powder containing 30 per cent was developed for use against plant diseases. This report records work on the persistence of etem on apple leaves and on scab control; that on disease control was inconclusive but the results are given now because further work is not contemplated.

The Method for Deposits.

Technically pure etem recrystallized from ethyl alcohol, m.p. 124–5°C, was used in tests, with and without plant material, to obtain a method suitable for deposits. The liberation of CS₂ on treatment with dilute mineral acid was unsatisfactory. Colours were given on the addition of cupric acetate or ferric chloride in ethyl alcohol to chloroform extracts of treated plant material, but these procedures were not considered sufficiently reliable. The recrystallized compound showed absorption peaks at 283 mμ (molecular extinction coefficient=18,800) and at 227 mμ (8,900), and use was made of the absorption at 283 mμ to determine deposits. Considerable interference from chloroform extractives of leaves was met at this wavelength, but could be almost completely eliminated without undue loss of etem by treatment of the plant extract with a suitable amount of Darco G60 decolorising charcoal (Fig. 1).

The method used for deposits was as follows. Fifteen discs, each 1 cm², were taken from leaves with the punch described by Kearns and Morgan (5). The discs were hot extracted with chloroform in a small percolation apparatus for five minutes, the extract was cooled, 0.1 g. of charcoal was added, the mixture was kept for 3 min. with agitation, and filtered through a plug of cotton wool. The filter was washed with chloroform, the filtrate was adjusted

*British Standards Institution common names.

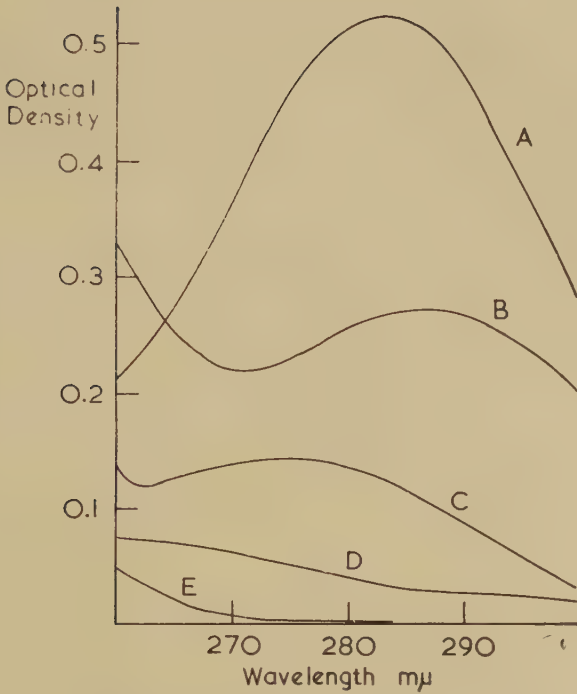


Fig. 1. Absorption curves of etem and decomposition products.

- A. Leaf extract+etem 6.5 $\mu\text{g./ml.}$ +charcoal.
- B. Primary decomposition product 5 $\mu\text{g./ml.}$
- C. Secondary decomposition product 5 $\mu\text{g./ml.}$
- D. Leaf extract+charcoal.
- E. Solvent.

to 15 ml. and the optical density was read at 283 $\text{m}\mu$, using a 1 cm. cell. The calibration line, allowing for determinations on untreated plant material, is shown in Fig. 2.

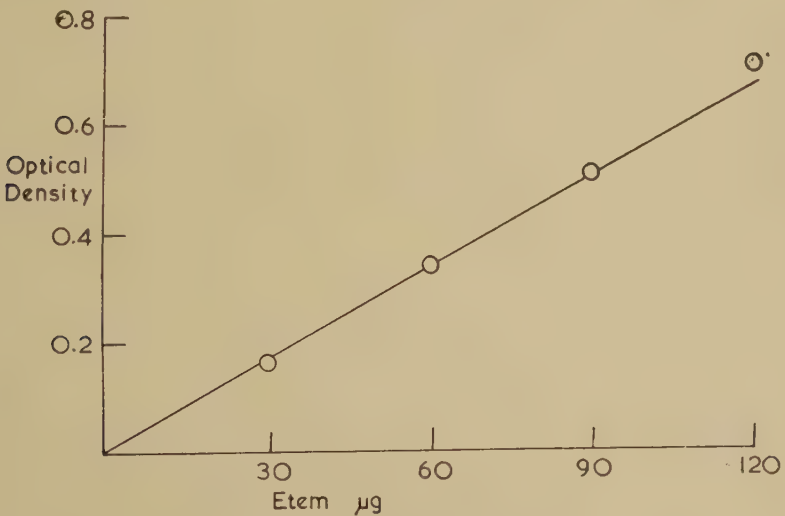


Fig. 2. Calibration line for etem.

The Rate of Loss of Deposits.

Worcester Pearmain apple trees in the orchard were sprayed to run-off on 11 May, 1959, using the proprietary dispersible powder at 2 lb./100 gal. (0.06 per cent active ingredient). After an interval of 3 weeks, during which 1.19" of rain fell, no etem could be detected on the leaves. Treatment was repeated on 2 June; three days later, following a further 0.50" of rain, the mean deposit of etem was 0.8 $\mu\text{g./cm}^2$.

The initial leaf deposits of 3-4 $\mu\text{g./cm}^2$ seemed to be reduced by the action of rain; another test was made to determine the effects of light and temperature. Apple seedlings (Worcester $\times$ Cox) raised in pots in the glasshouse were sprayed to run-off, using 0.06 per cent active ingredient, on 8 June. After the deposit had dried, some of the plants were placed outside and the others were kept in the glasshouse. Replicated determinations of etem on the leaves were made at intervals during three weeks. The mean results are shown in Table I.

TABLE I.
PERSISTENCE OF ETEM ON APPLE LEAVES IN THE OPEN AND UNDER GLASS.
($\mu\text{g./cm}^2$).

	<i>Initial</i>	<i>After 2 days</i>	<i>After 4 days</i>	<i>After 14 days</i>	<i>After 21 days</i>
In open ..	4.5	1.7	1.4	0.6	nil
Under glass	4.5	3.6	3.8	1.1	0.6

The temperature in the glasshouse during day-time varied between 60 and 80°F. The period of the test was bright, with 174 hours of sunshine; 0.26" of rain fell during the first two days, 0.06" between the second and fourteenth day and 0.71" during the last week.

The solubility of etem in water (up to 0.05 per cent at 20°C) no doubt accounted for the appreciable loss during the first two days in the open, but the decline in the deposits under glass suggested that degradation in the light was also taking place. Primary and secondary decomposition products were obtained from Messrs. Robinson Bros. and tested for their ultra-violet absorptions to ascertain to what extent, if present, they would interfere with the determination of etem. Their absorption curves are shown in Fig. 1. Each substance showed some absorption, indicating that the etem was probably over-estimated by the method used.

Orchard Evaluation.

For evaluation as a fungicide against apple scab caused by *Venturia inaequalis* (Cooke) Wint., the compound was used at a concentration of 0.06 per cent. This treatment was compared with captan (0.1 per cent active material), and a control. Sprays were applied to run-off using a handlance on 22 April, 11 May, 2 June and 30 June, 1959. One-tree plots were used of bush apple trees var. Worcester Pearmain, and treatments were replicated six times in a randomized block layout.

In the hot dry summer of 1959, scab incidence was extremely low. Mean percentage areas scabbed of leaf and fruit respectively at the time of assessment were etem : 0.009, 0.031 ; captan : 0.011, 0.003 ; control : 0.038, 0.055. No satisfactory evaluation of the compound as a scab fungicide could be obtained with so low an incidence of the disease. No phytotoxicity was observed throughout the trial.

Acknowledgments.

We are indebted to Messrs. Robinson Brothers, West Bromwich, for the samples of decomposition products, and to Mr. C. W. Harper for the spray applications.

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SPRAY APPLICATION PROBLEMS: LXIV. THE ABSORPTION AND MOVEMENT OF MERCURY IN PLANTS.

By J. A. PICKARD and J. T. MARTIN.

Because of its toxicological importance, work was continued during 1960 on the contamination of crops with mercury. Official recommendations for the use of organo-mercury foliage sprays define the conditions of application that should limit the residues of mercury on apples and pears at harvest to 0.1 parts per million (see p. 00) and state that, on present information, a residue not exceeding this amount should not present a hazard to the consumers of the fruits. The undesirability of mercury as a contaminant of foodstuffs is demonstrated by comparison of this unofficial tolerance of 0.1 p.p.m. with the official tolerances of 2 p.p.m. lead and 1 p.p.m. arsenic imposed for apples and pears ; the maximum amount of mercury considered safe on fruits is thus twenty times less than lead and ten times less than arsenic. Some countries indeed have imposed a zero tolerance for mercury in foods. Attention has therefore been given to the residues resulting from commercial-type applications of mercurial fungicides and to the degree of uptake and movement of mercury after coming into contact with plants. The analytical method used, which detects 1 μ g. of mercury (Hg), is described elsewhere (1).

The Entry of Mercury into Apple Fruits.

Work already reported (2,3) showed that after the application of phenyl-mercuric nitrate foliage sprays, Bramley fruits after harvest showed 0.05 p.p.m. Hg, distributed between the peel and flesh. Fruits from commercial orchards showed similar levels of contamination. Small fruits from one site contained 70-80 per cent of the mercury in the peel ; in large fruits from another site

almost 70 per cent was in the flesh. Mercury deposited as phenylmercuric acetate on the surfaces of fruits rapidly penetrated beyond the reach of surface washing, but appeared to be firmly held in, or just below, the peel. This result suggested that the mercury found in the flesh of the fruits was brought in from the leaves as the fruits increased in size. Work on apple leaves showed that mercury applied as phenylmercuric acetate penetrated quickly and tended to accumulate in the fibrous material associated with the vascular tissue, and indications that mercury applied as the acetate was absorbed by leaves and translocated were obtained in work on the coffee plant (4). Ross and Stewart in Canada, working with the acetate, suggested that mercury in apple fruits came from other parts of the tree (5) and in further work, in which fruits were protected from surface contamination during spraying, they obtained evidence of the translocation of mercury to the fruits as they matured (6).

During 1960, trials were made at Long Ashton to obtain further information on the movement of mercury into apple fruits. Although not aware of their work, our procedure closely followed that of Ross and Stewart. Bramley trees in the orchard were hand-sprayed to run-off with a proprietary phenylmercuric acetate solution (0.005 per cent Hg) with the addition of 3½ fl. oz. Ethylan C.P. wetter per 100 gallons. The first treatment was given soon after budburst and seven applications in all were made at intervals of three weeks up to mid-August. Immediately before the first application, fruit spurs selected at random were labelled and protected from the spray by enclosure in polythene bags which were removed as soon as the spray fluid had dried. The labelled spurs and, later, the developing fruits were similarly protected during each subsequent spray treatment. Fruits receiving the sprays and those protected were harvested in mid-October and kept in a cold store.

Each sample for analysis comprised four fruits selected at random. They were peeled and the cores were removed without attempting clean separation from the flesh; the calyx cups and small areas of peel around the petioles were included with the cores. The peel, cores and 100 g. portions of the macerated flesh from the four fruits were analysed separately. The results, as p.p.m. Hg., corrected for blank determinations on the peel, flesh and cores of untreated fruits, are shown in Table I. Reagent blanks were low and there were no

TABLE I.
MERCURY IN SPRAYED AND PROTECTED FRUITS.

Sample	Average weight of fruit (g).	Average %			Hg (p.p.m.)			Hg (p.p.m.) in whole fruit
		Peel	Flesh	Core	Peel	Flesh	Core	
	<i>Sprayed fruits</i>							
1	207	6.4	78.9	14.7	1.54	0.12	0.39	0.25
2	208	6.6	76.6	14.8	1.51	0.04	0.45	0.20
3	216	5.8	77.0	17.2	1.29	0.13	0.31	0.23
				<i>Mean</i>	<i>1.45</i>	<i>0.10</i>	<i>0.38</i>	<i>0.23</i>
	<i>Protected fruits</i>							
4	160	5.9	87.5	6.6	0.05	0.07	0.18	0.08
5	230	6.2	85.1	8.7	0.03	0.06	0.11	0.06
6	177	7.5	82.6	9.9	0.01	0.07	0.05	0.06
7	204	5.5	76.9	17.6	0.03	0.03	0.07	0.04
				<i>Mean</i>	<i>0.03</i>	<i>0.06</i>	<i>0.10</i>	<i>0.06</i>

indications that significant amounts of naturally-occurring mercury were present in the untreated fruits.

Mercury was present in the peel, flesh and cores of the fruits subjected to the sprays; little mercury occurred in the peel of the protected fruits but their flesh and cores showed definite contamination. The amounts and distribution of the mercury are shown in Table II.

TABLE II.
DISTRIBUTION OF MERCURY IN SPRAYED AND PROTECTED FRUITS.

Sample	Hg $\mu\text{g.}/\text{fruit}$			Hg $\mu\text{g.}/$ whole fruit	Hg %		
	Peel	Flesh	Core.		Peel	Flesh	Core
	<i>Sprayed fruits</i>						
1	20.2	19.3	11.8	51.3	39	38	23
2	20.8	6.4	13.7	40.9	51	16	33
3	16.0	20.8	11.2	48.0	33	43	24
Mean	19.0	15.5	12.2	46.7	41	32	27
	<i>Protected fruits</i>						
4	0.5	9.4	1.9	11.8	4	80	16
5	0.4	11.8	2.8	15.0	3	79	18
6	0.2	9.4	0.9	10.5	2	90	8
7	0.3	4.1	2.6	7.0	4	59	37
Mean	0.4	8.7	2.1	11.1	3	77	20

The total mercury in the protected fruits was 24 per cent of that in the sprayed fruits; 97 per cent of the mercury in the shielded fruits was present in the flesh and cores. The mean value of 0.4 $\mu\text{g.}$ Hg/fruit found in the peel of the protected fruits may have been due to penetration through the polythene bags. The flesh of the sprayed fruits contained 7 $\mu\text{g.}$ Hg/fruit more than that of the protected fruits; this excess probably arose from the penetration of some of the surface deposit to the zone immediately beneath the cuticle. The cores of the fruits subjected to the sprays contained six times more mercury than those of the protected fruits, doubtless due to the inclusion with the cores of the calyx and petiole depressions which tend to accumulate spray deposits. The comparison of the distribution of mercury in the sprayed and protected fruits fully supports the conclusion reached by Stewart and Ross that mercury applied to apple trees as phenylmercuric acetate is translocated into the fruits from the leaves.

Table I shows that the sprayed fruits contained 0.23 p.p.m. Hg, a value exceeding the unofficial tolerance and appreciably higher than the contamination found in commercial spraying. From experience with other crop protection chemicals this effect was considered to be due to the addition of wetter and to efficient hand spraying. Further work is planned on the influence of formulation on the levels of mercury deposit obtained on fruits and leaves.

The Contamination of Carrots grown in Mercury-treated Soil.

Other work at Long Ashton previously reported (3) showed that mercury was taken up by plant roots from nutrient solution and soil, but that little translocation to the aerial parts occurred. The possibility arose that edible root crops might become contaminated when grown in soil treated with

mercury preparations and this has been examined in collaborative work with the Rosewarne Experimental Horticulture Station of the National Agricultural Advisory Service.

Land previously under fallow at Rosewarne was fertilized, cultivated to a fairly fine tilth and divided into areas I and II, each approximately 1,000 sq. ft. Mercurial treatments, as used for club-root control, were applied to each area as follows :

Treatment A. Calomel (100 per cent) applied at 1 oz./150 ft. run.

B. Mercuric chloride, 1 in 2,000, watered in drills before sowing.

Each treatment was duplicated in each area. The treatments were made on 31 May, 1960, and carrot seed, variety Early Market, was sown along the treated rows on area I on the same day, and along the treated rows on area II on 27 July, thus allowing, on area II, an interval of two months between the soil treatment and sowing. Roots and tops taken from area I on 18 October, from area II on 7 December, and from untreated plots, were sent to Long Ashton for examination. The tops were washed and rinsed in tap and distilled water and the roots were washed and scrubbed before analysis. The results after correction for low reagent blanks are shown in Table III.

TABLE III.
MERCURY IN CARROTS GROWN IN MERCURY-TREATED SOIL.

Treatment	Area I		Area II
	<i>Tops</i>	<i>Roots</i>	<i>Roots</i>
A Calomel	<0.01	0.03	<0.01
	<0.01	0.01	<0.01
	<0.01	0.01	—
B Mercuric chloride	0.02	0.08	<0.01
	0.02	0.03	<0.01
	0.02	0.04	—
C Untreated	<0.01	<0.01	<0.01
	—	<0.01	<0.01

Only the roots of plants from seed sown immediately after mercuric chloride treatment showed noticeable contamination (mean 0.05 p.p.m. Hg). The roots from treatments A and B on area I were comparable in weight (2 oz. per root); hence the higher level of contamination of the roots from the B treatment was not due to restricted growth. The distribution of mercury in the roots was not determined; contamination by attached soil was not thought likely. Germination was suppressed when the seeds were sown immediately after treatment of the soil with mercuric chloride, but on the other treated plots the seeds germinated normally.

5.5" of rain fell during the interval between the soil treatments and sowing on area II, and the roots then showed no differences from those from untreated soil. Because of the absence of contamination in the roots, the tops were not examined.

The values for apparent mercury recorded in the analyses of the roots from area II and from untreated soil were slightly but consistently higher than

the very low values given by the reagents alone. Small amounts of naturally-occurring mercury may be present in the roots but this is not easy to substantiate. Work on the risks of contamination of other root crops grown in mercury-treated soil will be reported later.

Summary.

Evidence is presented that mercury, applied to Bramley apple trees as phenylmercuric acetate, passes into the fruits by translocation from the leaves. Carrot roots from plants raised from seed sown immediately after soil treatment with mercuric chloride contained 0.05 p.p.m. Hg; roots from plants grown in soil treated with calomel were not contaminated.

Acknowledgment.

We are greatly indebted to Miss A. M. Williams of Rosewarne for conducting the soil experiment and supplying the carrot samples.

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SPRAY APPLICATION PROBLEMS: LXV. SPRAY DEPOSITS, TECHNIQUES AND MATERIALS FOR THE CONTROL OF APPLE APHIDS AT THE 'GREEN CLUSTER' STAGE.

By N. G. MORGAN.

Introduction.

Two of the major post-war developments in fruit-spraying techniques, the substitution of winter washes by spring and summer sprays and the use of small (low) volume spraying, have increased the need for more exact information on the biological requirements of spray applications. The biological requirement of winter spraying for the control of apple aphids, for example, might be simply specified as the wetting of all the overwintering eggs on the tree with tar oil, DNOC-petroleum oil emulsion, or other winter wash whose efficiency had been established. This simple requirement could only be assured by drenching the trees with large volumes of the dilute emulsion.

The biological requirements of spray-applications in the spring, however, are not so simply definable. Variations in the time of hatching of the different aphid species, in their habits and in the mode of action of the different spray materials available for spring application, and the choice between using large-volume or small-volume spraying techniques, point to the need for more precise determination of the factors involved in the control of these insects.

Two important species of aphid attacking the apple are the Oat-Apple or Apple-grass aphid, *Rhopalosiphum insertum* (Wlk.) and the Rosy Apple aphid, *Sappaphis plantaginea* (Pass.). In most seasons the peak period of hatching of the first species from the winter eggs coincides roughly with the 'bud burst' stage of the variety Worcester Pearmain and with the 'mouse ear' stage of Cox's Orange Pippin. At this time the newly-hatched aphids collect in an exposed position on the outside of the buds, where they are vulnerable to spray materials. Kearns and Morgan (1) have shown that good control can be obtained at this stage by air-flow mist spraying of dilute DDT/BHC. Later, at the 'green cluster' stage, when the rosette leaves have opened, both Oat-apple and Rosy Apple aphids can be found feeding in a protected position between the closely packed flower buds. At the 'pink bud' stage the flower stalks have separated and the aphids are again exposed.

At first sight it would appear that the most appropriate spraying times for the control of these insects is at the 'bud burst' or 'pink bud' stages, when they are exposed. For biological and practical reasons, however, it is essential that spray applications should be made at the 'green cluster' stage. First, combined spray materials must be applied at the 'green cluster' stage for the control of other important pests such as Apple Sucker, *Psylla mali* (Schr.) and the caterpillars of the Winter Moth, *Operophtera brumata* (L.) which hatch later than the Oat-apple aphid. Secondly, on some plantations it is rarely possible for large acreages of apples to be sprayed within the short period represented by the 'pink bud' stage. At the 'green cluster' stage, the aphids present in the clusters are stem mothers which, if not controlled at this stage, later produce large numbers of young. These young aphids begin feeding and, in the case of the Rosy Apple aphid, cause considerable leaf curl and also damage to the crop.

Experiments were carried out in order to establish some of the conditions necessary for aphid control at this important stage.

SPRAY DEPOSITS AND APHID CONTROL.

An experiment was made in 1957 to assess the deposits obtained by different methods of spray application at the early and full 'green cluster' stages and to determine the efficacy of these deposits in controlling the Oat-apple aphid. Large-volume hand-lance spraying was compared with mist spraying at two rates of application per acre.

Experimental.

A combined emulsion, containing 0.05% technical DDT and 0.0125% gamma BHC, prepared by dissolving the insecticides in solvent naphtha and emulsifying with sulphite lye, was used with added wetting agent (0.025% dioctyl sodium sulposuccinate). The hand spraying was done with a 3-nozzle

lance at 300 p.s.i. flow pressure, each tree being sprayed from several different directions. Approximately 300 gallons per acre were used to ensure the full cover of all parts of the tree until run-off of surplus liquid occurred.

The experimental air-flow machine used for the mist spraying was identical in basic design with that described by Kearns and Morgan (2) but was fitted with a fish-tail air outlet trunking, with internal guide vanes directing the air through a rectangular orifice $72'' \times 9''$. On each side of this orifice was fitted a $\frac{3}{4}''$ bore copper tube with 15 nozzles ($\frac{1}{4}''$ B.S.P.) spaced $9''$ apart and angled at 45° to the air stream; the nozzles were fitted with discs of $3/64''$ orifice diameter. At a travelling speed of $2\frac{1}{2}$ m.p.h. the approximate application rates were 60 gallons per acre with 15 nozzles in operation and 112 gallons per acre with 30. The machine was driven along the rows so that the trees were sprayed from two opposite sides, with the trunking at 80° to the axis of travel and at 35° to the horizontal.

The spraying was done on a block of Cox's Orange Pippin trees with Worcester Pearmain pollinators. The plot was originally planted at $15' \times 15'$ but later thinned diagonally to 96 trees per acre. 6 Cox and 6 Worcester were used for the hand spraying, 13 Cox and 5 Worcester for the 60 gallon per acre mist spraying, and 18 Cox and 6 Worcester for the 112 gallon per acre mist spraying. At the time of spraying on 1st April the Cox were at the full 'green cluster' stage and the Worcester at 'mouse-ear'.

At the 'bud burst' stage of the Worcester trees large numbers of fruit buds infested with aphids had been labelled at random throughout the treatment blocks and also on trees to be left unsprayed. After spraying, when the spray deposits had dried, 20 labelled trusses were removed from each variety on each block and the leaves and flower buds analysed separately to ascertain the level of the DDT deposits.

The remaining labelled trusses were examined on 10th and 16th April for the presence of aphids. Between 60 and 335 trusses were examined on each block.

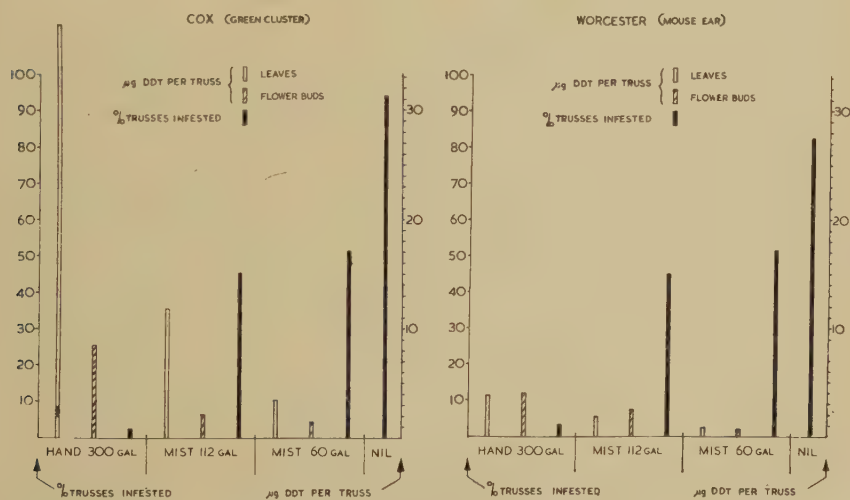


Fig. 1. Methods of application, DDT deposits and Oat-apple aphid infestation.

(a) Cox's Orange Pippin ('green cluster'); (b) Worcester Pearmain ('mouse ear').

Results.

The results (Fig. 1) show that the biological efficiency of the hand-lance spraying was superior to that of both the mist sprayings. The larger-volume mist spraying gave slightly better control than the smaller one. Aphid control on the Worcester trees was little different from that on the Cox.

The spray deposits, expressed as the weight of DDT per truss, show that more material was deposited by the large-volume hand spraying than by the mist spraying, the difference being greater at the 'green cluster' stage of Cox than at the 'mouse-ear' of Worcester. The difference between the deposits from the two mist applications was also greater at the 'green cluster' stage.

At the 'mouse ear' stage the amounts of DDT recovered from the leaves were little different from those recovered from the flower buds, but at the 'green cluster' stage these amounts were greater, though the difference decreased as the volume of liquid applied per acre decreased.

Discussion.

Although the levels of BHC deposits were not assessed, it is fair to assume that the DDT levels give a measure of the amounts of both active materials deposited. It is not known, however, what separate contribution to aphid control was made by each material.

The assessment of aphid infestation was based on the proportion of clusters infested and not on the number of aphids per cluster before and after the spraying. The DDT analyses were carried out on the bulked portions of 20 trusses per treatment. Therefore, the partial control shown by the mist applications indicates that the spray deposits were completely effective on some of the clusters, and not that they were partially effective on all the clusters examined.

The amount of DDT deposited on the Cox flower-truss leaves by the mist application at the 60 gallon rate, which showed only partial control, was approximately equal to that given on the Worcester leaves by the almost completely effective hand spraying. Furthermore, the amount deposited on the Cox leaves by mist application at the 112 gallon rate, which also showed only partial control, was three times that on the Worcester leaves sprayed by hand. This indicates that aphid control was obtained not by material deposited on the leaves but by that on the flower buds.

The superior aphid control obtained on the hand sprayed trees appeared, at first sight, to be due to the greater amounts of insecticides deposited on the flower buds by this method. Observations on the position of the aphids and on the behaviour of the spray liquid at the time of spraying, however, indicated that the liquid soaked into the truss between the flower buds and reached the aphids in this way. On most of the mist-sprayed trusses, examined at the same time, this did not occur because the volume of liquid deposited was insufficient.

APPLICATION OF DDT AND BHC TO INDIVIDUAL CLUSTERS.

Experimental.

A small-scale experiment was carried out in 1958 in order to observe the effect on the Oat-Apple aphid of insecticides applied to individual clusters at the early 'green cluster' stage with and without wetting agent.

A total of 837 infested buds of the variety Lord Lambourne were first labelled at the 'bud-burst' stage. At early 'green cluster', when all the rosette leaves had emerged from the bud but were not completely separated from the flower buds, dilute DDT and BHC emulsions, prepared as in the previous experiment, were applied with an artist's brush to all exposed parts of each cluster. Approximately 100 clusters were used for each treatment and no attempt was made to standardize the volume applied. The materials used in the separate treatments were DDT, BHC, and combined DDT/BHC emulsions, applied both alone and with succinate wetting agent.

Soon after the materials thus deposited had dried, 10 of the clusters in each treatment in which DDT was used were removed and dissected for separate DDT analyses of leaves and flower buds. Counts were made at the 'pink bud' stage of the proportion of the remaining clusters infested

Results.

The results given in Fig. 2 show that in each case aphid control was superior on the clusters treated with insecticide with wetting agent to that on the clusters treated with insecticide alone. This effect was most marked with the combined DDT/BHC emulsion in which the concentration of each insecticide was half that in the separate emulsions. BHC (0.0125% gamma) with wetting agent (0.025% dioctyl sodium sulphosuccinate) gave complete control and BHC alone gave control approximately equal to that of DDT (0.05%) with wetting agent.

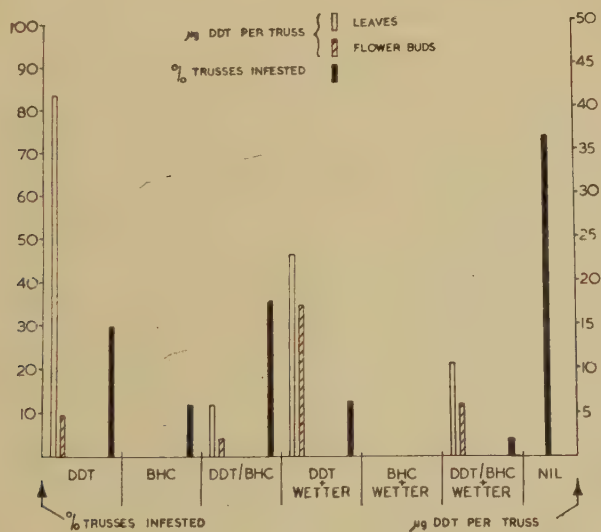


Fig. 2. DDT deposits and Oat-apple aphid infestation. Spray materials applied by brush to individual clusters on variety Lord Lambourne at early 'green cluster'.

The DDT figures show that in each treatment more material was deposited on the leaves than on the flower buds and that more was deposited on the flower buds by the emulsions to which the wetter had been added than by the emulsions alone.

Discussion.

It was evident, by observation at the time of application, that most of the emulsions without wetting agent ran off the flower buds and collected mainly on the upper surfaces of the leaves, whereas those with wetting agent spread over all parts of the cluster, including the surfaces between the flower buds.

Apart from showing the superior control obtained by the addition of the succinate wetter, the results also suggest that the contribution of the BHC to aphid control was greater than that of the DDT at the concentrations used, since the BHC alone gave better control than DDT alone; it was in fact as good as DDT with wetter. The indications are that the BHC was either more toxic to the aphids or that it exerted its lethal effect at some distance from the site of deposition. The best control was given, however, by the BHC with wetter.

The good control obtained by the application of the combined DDT/BHC emulsion with wetting agent indicates that the amount of insecticide required for control is small, provided it is deposited at the correct site.

SPRAY MATERIALS AND TECHNIQUES FOR APHID CONTROL.

A field experiment was made in 1960 to determine the effect on aphids at the 'green cluster' stage of different insecticides applied by different spraying techniques.

Experimental.

Four materials, pyrethrum, combined DDT/BHC, malathion, and the systemic demeton-methyl were each applied in three ways: large-volume hand lance spraying of dilute material, small-volume spraying of dilute material and, except for demeton-methyl, small-volume spraying of the materials at five times the concentration. A succinate wetter was used throughout at 0.025%.

The hand spraying was carried out as described in the first experiment. The small-volume spraying was done with a tractor-mounted assembly consisting of a tank, feed pump and a large air compressor, belt-driven from the tractor pulley, supplying air at 75 p.s.i. to six swirl-mixing twin-fluid nozzles designed and described by Cobbald (3). These nozzles were approximately equally spaced on a 10 ft. vertical mast and adjusted to spray at right angles to the travel direction on one side of the tree-row at a time.

The spraying was carried out on 19th April, 1961, on the same plot used for the first experiment described above, when the Cox were at the 'green cluster' stage and the Worcester at 'mouse-ear'. Each treatment block, and an unsprayed block, consisted of four Cox and two Worcester trees. The experiment was repeated on 26th April on a Worcester plot when this variety was at the full 'green cluster' stage; in this case each treatment block consisted of between ten and twenty trees. On each plot the volume of liquid applied per acre was 300 gallons for the large-volume spraying and 64 gallons for the small-volume. The method of assessing aphid infestation was identical with that in the first experiment.

Results.

The results given in Fig. 3 show that with each material the large-volume hand spraying gave the greatest reduction of infestation, the order of efficiency being : demeton-methyl>malathion>DDT/BHC>pyrethrum.

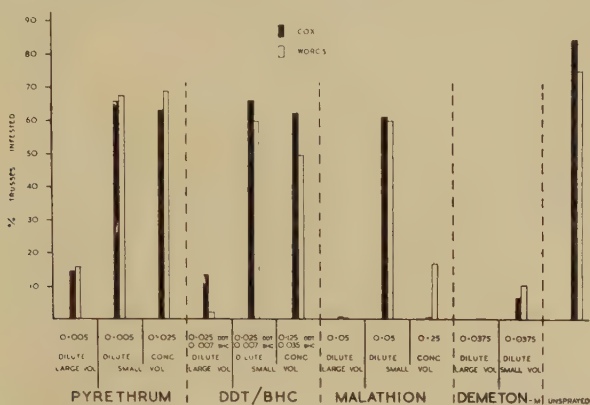


Fig. 3. Materials and methods of application for control of apple aphids. Cox at 'green cluster', Worcester at 'mouse ear.'



Fig. 4. Materials and methods of application for control of apple aphids. Worcester at 'green cluster'.

The small-volume application of dilute demeton gave results far superior to the small-volume applications of the other three dilute materials. Increasing the concentration of the materials five times made no significant difference to the efficiency of the small-volume application of pyrethrum and DDT/BHC but made a marked difference to that of the malathion.

The level of infestation on the unsprayed trees on the Worcester plot (Fig. 4) was only half that on the Cox plot, but the results of the treatments on the two plots are similar. The main differences are the complete control given on the Worcester plot by the large-volume application of dilute, and the small-volume application of concentrated malathion, and the small-volume application of dilute demeton.

A small-scale test was made of the two methods of application using a water-soluble fluorescent dye. Dissection of sample trusses showed that the spray liquid applied in large volumes had completely wetted all parts of the truss, including the flower stalks, while that applied by the small-volume method appeared in the form of small separate deposits on the outside surfaces of the trusses only.

Discussion.

Interpretation of the results must take into account the conditions and limitations of this type of spraying experiment. The plot, and therefore each treatment block, was small, and replication of treatments was not possible. On the other hand, the large number of infested blossom trusses labelled before and examined after the spraying, between 300 and 400 per treatment, helped to establish the validity of the effects obtained. The treatment blocks were close to each other, with no intervening guard trees, but the possible overlapping effects of spray drift were minimized by arranging the large-volume spraying downwind of the small-volume, the concentrated insecticide spraying downwind of the dilute and the demeton-methyl and malathion downwind of the pyrethrum and DDT/BHC.

It can be argued that the spray deposits on a few experimental trees would not correspond to the accumulated deposits resulting from the spraying of a large area of trees. It is, however, unlikely that the efficiency of the spray materials that failed to control the aphids, when applied to the trees from two sides at close range, would be increased by drift from one direction at longer range.

The results emphasize the need for considering the biological requirements of spray application. It is clear that aphid control at the 'green cluster' stage cannot be assured merely by increasing the concentration of insecticide in reciprocal proportion to the reduction in volume of liquid used per acre. This procedure was shown to be unnecessary with demeton-methyl, it failed with pyrethrum and DDT/BHC, and was justified only with malathion.

From the practical point of view, each of the four materials gave good control of apple aphids when applied at this stage in large volumes in combination with an efficient wetting agent. The small-volume spraying technique, however, produced an initial distribution of spray liquid which was insufficient to give control unless the active principle was redistributed to the correct site. This occurred only with the truly systemic material demeton-methyl, by translocation, and with the concentrated malathion, presumably by diffusion.

Summary.

Large- and small-scale field experiments have shown that good control of apple aphids at the 'green cluster' stage by dilute pyrethrum, DDT, combined DDT/BHC or malathion was obtained only by the application of large volumes of spray liquid to which an efficient wetting agent had been added; small-volume application of these materials gave poor control. A five-fold increase in concentration of the insecticides applied in small volumes also failed, except in the case of malathion. Small-volume application of dilute systemic demeton-methyl gave good control.

Apple aphids at this stage are in a protected position between the closely packed flower buds, and control depends on the deposition of active material at this site. The large-volume applications deposited sufficient spray liquid on the flower buds for the insecticides to reach this site by the spreading action of the added succinate. The initial distribution of spray liquid deposited by the small-volume applications was, however, insufficient to give control unless the active principle was redistributed to the correct site : this occurred only with the systemic demeton-methyl, by translocation, and with the concentrated malathion, presumably by diffusion through the tissues of the flower truss.

Acknowledgments.

Grateful acknowledgments are due to Dr. J. T. Martin and his colleagues for the DDT analyses, to Mr. T. E. Cobbald for help with the compressed-air spraying equipment, to Mr. J. H. Russell for assistance with the spray applications and to Miss B. Bird for work on the biological assessments.

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SPRAY APPLICATION PROBLEMS : LXVI. THE DISTRIBUTION OF COPPER OBTAINED ON TOBACCO IN NORTH BORNEO WITH A MODIFIED SHOULDER-MOUNTED MIST-BLOWER.

By ANN R. TAPSCOTT and H. R. MAPOTHER.

Introduction.

Ring spots due to Frogeye Disease (*Cercospora nicotianae* Ell. & Ev.) cause serious losses to growers of cigar-wrapper leaf in North Borneo, as the affected leaves fail to reach the high standard demanded of wrapper tobacco (1, 2). The disease can be controlled by fungicides, but the routine estate spraying programme has given uncertain results. This was believed to be primarily due to faulty techniques of application, as leaf samples taken from newly-sprayed tobacco have frequently revealed little or no deposit.

The tobacco is planted in raised beds 39" apart, each bed consisting of two staggered rows 15" apart, with 18" between plants. At maturity the crop is up to 8' high. As the land is impeded in places with large boulders and the remains of jungle trees, the use of hoses is not practicable and only machines that can be carried by the operator are feasible. Standard equipment on the estates consists of knapsack pneumatic sprayers, filled at central power-operated pumps.

Erratic results, probably due to the masking effect of the dense foliage, were also obtained at Long Ashton in 1959 when machines of this type were tested on potted tobacco set out at estate planting distances. Better performance was promised by a shoulder-mounted mist-blower which was able to penetrate the foliage by reason of the turbulence of the air stream. Experiments were therefore made with a commercial blower* of this sort at Long Ashton to find the type of nozzle assembly giving the best results. This machine, as subsequently modified, was used in small-scale and field trials with tobacco in North Borneo in 1960.

Experimental.

To obtain preliminary data on spray distribution, artificial plants, constructed of bamboos and Sisalkraft paper, were set out at Long Ashton to simulate a tobacco bed. This device proved sufficiently satisfactory for making tests on various designs of nozzle. The distribution and levels of spray deposit on the target were examined using a readily-assayed fungicide containing 3% copper. To permit repeated use of the artificial plants, spray deposits were measured on twin-layered discs ("Capseals"), $\frac{3}{4}$ " in diameter, attached to the leaves by a dab of Bostik applied to the card side. By inserting the point of a knife the front vinylite layer could readily be stripped with tweezers, thus discarding the back layer contaminated by contact with the leaf.

Nozzle Development.

The requirement was a nozzle that would give reasonably uniform cover on plants at all stages of growth when directed by an operator walking forward in the alley between two tobacco beds. As the type of nozzle supplied with the

TABLE I.
COPPER DEPOSITS ($\mu\text{g./cm.}^2$) COLLECTED ON DISCS: LONG ASHTON.
Single passage of machine. Spray concentration 3.0% Cu.

Discs attached to bamboos set 18" apart				Discs attached to artificial tobacco leaves				
Height above ground (in.)	Position in the row			Height above ground (in.)	Front Row		Back Row	
	Front	Middle	Back		Upper surface	Under surface	Upper surface	Under surface
0	11.2	25.5	7.0	Base	7.9	1.3	5.6	0.9
6	9.6	32.7	8.7					
12	4.4	26.6	7.9	12	7.7	6.9	1.2	0.7
18	1.5	16.4	5.9					
24	3.8	10.8	9.4					
30	1.4	4.6	4.7	30	6.1	1.2	1.1	0.5
36	0.7	2.7	1.8					
42	0.6	1.7	1.1					
48	0.6	0.4	1.4	48	2.3	2.6	0.4	0.4
54	0.6	0.6	0.4					
60	0.3	0.3	0.4	60	0.3	0.4	0.5	0.5

*Motoblo (K.E.F. Ltd., Kent). 1958 Model: 100 c.c. 2-stroke engine: Maximum air-speed 240 m.p.h.

PLATE II



Fig. 1. Four-nozzle assembly.



Fig. 2. Metering plug, manifold, connecting tube and shear tube.

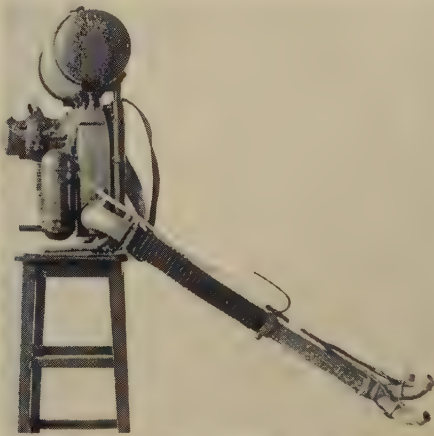


Fig. 3. Complete machine.



Fig. 4. Sprayman about to enter alleyway between beds of tobacco 8 ft. high.



Fig. 5. Diffuser in use on plants 18 in. high.

machine did not appear to be the most suitable for this purpose, a new type of plastic nozzle assembly (Plate II, Fig. 1) was designed. It comprised two pairs of diverging up-tilted air nozzles, the lower pair inclined at 10° and the upper at 60° to the horizontal. Each nozzle was fitted with a shear-tube of $\frac{1}{8}$ " bore with the end cut at a slope of 30° (Fig. 2); the use of tubing of this diameter was found to eliminate surging of the spray fluid. The liquid was taken through a metering plug to a manifold with a feed to the shear-tube in each nozzle. The complete assembly weighed $\frac{3}{4}$ lb. and could be held without difficulty in front of the sprayman; in practice it was used about 6" above the ground, i.e. at bed level.

Spray deposits typical of a number of test runs of the machine on the artificial plants at Long Ashton are given in Table I.

Spray Trials in North Borneo.

The same type of machine and nozzle assembly employed at Long Ashton was used in field trials with tobacco in North Borneo in 1960, and found satisfactory for plants over 18" high; for younger plants the diffuser attachment supplied with the machine was found more suitable.

Visual records of the distribution of spray deposits on the crop were obtained by the application of 0.1% suspensions of the fluorescent tracer Saturn Yellow. Deposits were classified in categories ranging from 8 (heavy and uniform) to 0 (nil). Check analyses from 0.1% Cu sprays containing tracer gave the following values for samples of uniform deposits :—

	<i>Tracer</i>	<i>Copper ($\mu\text{g./cm.}^2$)</i>
Heavy	(Category 8)	10.2—34.0
Medium	(" 6)	2.2—4.4
Light	(" 4)	0.3—0.4

The tests with the fluorescent tracer showed spray distribution to be satisfactory. Further experiments to determine the levels of spray deposit were made in the following trials :

1. Application of 0.1% Cu spray by the diffuser to crops not exceeding 18" in height.
2. Application of copper sprays (0.1 and 1.0% concentration Cu) by the nozzle assembly to crops over 18" in height.
3. Field-scale (1 acre) application of a spray of copper oxychloride (0.1% Cu) and 0.025% Ethylan C.P. wetter from the seedling stage until leaf maturity.

Trial 1. Diffuser (Table II). The spray deposits of copper fungicide were collected on discs attached to the leaves of plants 4", 12" and 18" high, in the row adjacent to the alley-way and in the second row. The discs were placed on only one surface, as the fluorescent tracer tests had shown that the spray deposit was uniform on upper and lower surfaces.

Trial 2. Four-nozzle assembly (Table III). The plants were sprayed with one pass of the mist-blower. Discs were attached to the upper and lower surfaces of every second pair of leaves of three plants selected at random in each of the two rows in the bed.

TABLE II.

COPPER DEPOSITS ($\mu\text{G.CU}/\text{CM}^2$) ON DISCS IN NORTH BORNEO TRIAL WITH DIFFUSER.

Spray concentration 0.1% Cu.

Leaf No. from base	Height of plant						Means
	4 in.		12 in.		18 in.		
	Row ad- joining alley	2nd row from alley	Row ad- joining alley	2nd row from alley	Row ad- joining alley	2nd row from alley	
1	2.2	1.2	3.9	1.5	1.7	0.6	1.9
2	5.1	1.9	1.1	3.0	3.5	1.8	2.7
3			1.2	3.0	2.5	3.4	2.5
4			2.7	2.2	1.2	0.8	1.7
5			0.7	4.1	2.2	4.5	2.9
6					7.2	2.6	4.9
7					1.1	1.0	1.1
Means	3.7	1.6	1.9	2.7	2.8	2.1	

TABLE III.

COPPER DEPOSITS ($\mu\text{G.CU}/\text{CM}^2$) ON LEAVES IN NORTH BORNEO TRIAL WITH FOUR-NOZZLE ASSEMBLY.

Means of two determinations on each of three plants.

Leaf No. from base	Row adjoining alley		Second row from alley	
	Upper Surface	Lower Surface	Upper Surface	Lower Surface
<i>Spray concentration 0.1% Cu</i>				
1	0.2	0.9	0.2	0.6
2	0.3	0.7	0.4	0.2
5	0.2	0.5	0.7	0.4
6	0.4	0.5	0.3	0.5
9	0.2	0.5	0.3	0.2
10	0.2	0.3	0.3	0.2
13	0.2	0.3	0.2	0.3
14 (top)	0.3	0.3	0.2	0.4
<i>Spray concentration 1.0% Cu</i>				
1	1.4	3.2	8.7	0.4
2	1.8	2.7	4.7	1.7
5	1.1	0.5	0.8	2.2
6	0.7	1.7	0.6	1.7
9	1.0	0.6	1.9	0.5
10	0.6	1.7	1.1	0.6
13	0.8	1.3	1.4	0.8
14 (top)	0.8	0.6	1.1	0.9

Trial 3. Spraying at intervals during growth of the crop (Table IV). The first three applications of the copper spray at 0.1% Cu were made by means of the diffuser. The four-nozzle assembly was used from the fourth to the tenth application (the fifteenth leaf).

Samples of the upper and lower leaf surfaces of the leaves were taken directly after spraying. The spray deposit consisted almost entirely of the last

application, with very low levels from previous applications. These very low levels were due to the rapid and extensive leaf growth between spray applications.

TABLE IV.

COPPER DEPOSITS ($\mu\text{g. Cu/cm.}^2$) ON LEAVES IN NORTH BORNEO TRIAL USING SUCCESSIVELY
DIFFUSER AND FOUR-NOZZLE ASSEMBLY.

Spray concentration 0.1% Cu. Means for two leaves.

Nozzle	Days from first spray application	Leaf No. from base	Row adjoining alley		Second row from alley	
			Upper surface	Lower surface	Upper surface	Lower surface
Diffuser	0	1	0.2	0.3	0.3	0.4
"	6	1 & 2	0.7	0.3	0.3	0.6
"	12	3	0.6	0.2	0.4	0.4
Four-nozzle assembly	19	4 & 5	0.4	0.6	0.3	1.0
"	25	6	0.6	0.4	0.3	1.1
"	30	8	0.5	0.9	1.0	1.3
"	34	8	0.4	0.3	0.3	0.4
"	38	10	1.1	0.6	0.3	0.4
"	44	12	0.2	0.2	0.2	0.1
"	56	15	0.1	0.1	0.2	0.3

It will be noted from these trials that the levels of copper deposit obtained with the diffuser on discs in the small-scale trial (Table II) are at least double those obtained on the leaves in the field trial (Table IV). The latter values are probably more representative of those likely to be obtained in practice. The level of copper deposit obtained from an application of 0.1% Cu with the nozzle assembly is generally of the order of 0.5 $\mu\text{g./cm.}^2$ on each leaf surface (Table III). The application of the same spray at ten times the concentration increased the deposit 2-5 times (Table III). The levels of spray deposit obtained with the four-nozzle assembly in the field trial were of the same order on the leaves as on the discs. They became lower after the tenth leaf, i.e. when the plants were about 5'-6' high; at this height the leaves are decreasing in size towards the flower spike.

Spraying Technique.

A number of further trials were made to ascertain the most effective procedure for using the diffuser and the four-nozzle assembly in routine spraying. Detailed results are not given in this paper, but the conclusions reached may be summarized as follows:

(1) The sprayman should not walk faster than 1 m.p.h. along the alley between two beds.

(2) The diffuser, with a throughput of $\frac{3}{4}$ pint per minute, was best used on plants up to 18" high; the optimum distribution of the spray was obtained by holding the diffuser $2\frac{1}{2}'$ above the ground, directing it so that it impacted $10'$ away. Only one bed could be sprayed per pass.

(3) The four-nozzle spray assembly, with a throughput of 1 pint per minute, gave an adequate coverage of two beds per pass.

Acknowledgments.

The authors thank Professor Kearns for his interest and advice in their work; they acknowledge the great help given in the design and construction of spray equipment by Mr. T. E. Cobbald. They also thank Mr. J. Rowse, Manager, and Mr. B. H. Hucker, Research Assistant, Darvel Estates, and Mr. A. G. Robertson, Research Officer, Imperial Tobacco Co. Ltd., for their willing help and co-operation.

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STUDIES ON PLANT CUTICLE: IV. THE LEAF CUTICLE IN RELATION TO INVASION BY FUNGI.

By MARGARET F. ROBERTS,¹ J. T. MARTIN and O. S. PERIES².

The reason for the differing susceptibilities of closely-related plants to fungal diseases constitutes one of the most intriguing problems in plant pathology. One variety may be severely damaged but another, equally exposed to infection, remains relatively unaffected. Most of the hypotheses put forward in explanation relate resistance to an effect of the plant upon the fungus, but the underlying basis of immunity remains obscure.

The surface protective layers of leaves are likely to play an important part in host-pathogen relationships. To gain access to leaves, many fungi must penetrate the cuticles. These appear to provide a mechanical barrier to invasion; young leaves are more readily attacked by fungi than are old leaves whose cuticles are thicker. The surface layers possibly protect the plant in other ways. Leaf secretions may affect the germination of fungus spores on the surface, or substances antagonistic to fungi may occur within the cuticles. Other work suggests that the degree of infection may depend upon the nutritional requirements of the fungus and the extent to which these are provided by the plant. Immunity probably results from a combination of circumstances disadvantageous to the fungus rather than from any single factor. To gain further insight into the problem, the levels of deposition of cuticle components in susceptible and resistant leaves and the effect of the fungus and the cuticle upon each other have been examined; the results are given in this progress report.

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Invasion of the Cuticle.

Special attention was given to powdery mildews ; these fungi invade only the surface layers of leaves and so are directly subjected to any protective influences that the cuticles may show. In making its way into the leaf, the fungus passes through zones of intermingling substances. The spore alights, usually upon a waxy covering. The cuticle is penetrated by an infection thread formed from an appressorium on the surface ; as the thread proceeds inwards, it enters a zone of cutin impregnated with more waxy material. On leaving the cutin, the thread passes through a layer rich in pectin and finally breaks through the cellulosic outer wall of the epidermal cell.

The surface waxy covering of leaves is usually composed of two fractions ; a hard or true wax consisting of long-chain paraffinic compounds and a soft wax or oil. An acidic portion occurs in the coverings of some leaves. Our tests have indicated that the true wax predominates near the surface and that the oil and acidic fractions are more deeply seated in the cuticles. Little is known of the chemistry of cutin ; recent work in Australia and S. Africa has shown that it is a poly-ester readily resolved by saponification into its constituent cutin acids.

The Determination of Cuticle Components.

The components were determined by the methods fully described elsewhere (1). Surface waxes were removed by immersing leaves of known total surface area (1000-2000 cm²) individually in solvent at room temperature. Chloroform was the most satisfactory solvent ; the amounts of waxy materials recovered from apple leaves by four successive immersions, each of 10 sec., in different solvents are shown in Fig. 1.

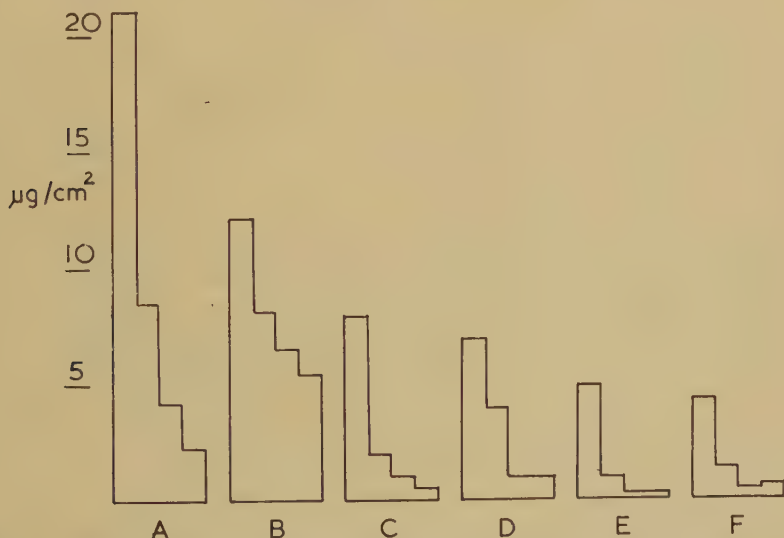


Fig. 1. Waxes obtained by successive immersions in : A chloroform ; B ether ; C carbon tetrachloride ; D benzene ; E petroleum ether ; or F hexane.

Acidic substances were removed from the waxy material by titration from ether solution with dilute alkali, and the true wax was separated from the

oil by partition between *n*-heptane and methanol. Discs punched from the leaves after immersion were further extracted with chloroform to give the absorbed waxes; they were then methanol-extracted to remove pigments and treated with oxalate solution to yield the cuticular membranes from the upper and lower surfaces. After treatment of the membranes with dilute acid and alkali solutions, cutin was determined by loss of weight on saponification or by the recovery of the ether-soluble cutin acids.

The small amount of material obtained from the fourth immersion in solvent, in comparison with the much larger amount of absorbed material, indicated that little leaching from within the leaves occurred during the separation of the surface waxes, but there was no assurance that the absorbed waxy substances came solely from the surface layers of the leaves.

The Cuticle as a Barrier to Invasion.

Leaves of increasing age were examined to ascertain the levels of cuticle components with which an invading fungus had to contend. The evergreen *Euonymus japonicus* (Linn.) is attacked by a powdery mildew which overwinters on the fully grown leaves. Analyses of leaves, in the spring, gave the results shown in Table I.

TABLE I.
CUTICLE COMPONENTS OF *E. JAPONICUS* LEAVES.
($\mu\text{g./cm}^2$).

				Leaf from apex of shoot					
				1	2	3	4	5	6
Waxes :	Surface	..	..	22	14	11	7	12	13
	Absorbed	..	..	77	57	36	50	33	95
Cutin :	Upper surface	..	..	219	302	382	390	483	643
	Lower surface	..	..	185	233	320	313	463	540

Leaves 1-5 were on new shoots; leaf 6 was the last-formed leaf of the previous year's growth. The smallest new leaves that could be examined (leaf 1) carried as much waxy material as old leaves and cutin deposits of about 200 $\mu\text{g./cm}^2$. If the cutin provides a barrier, this would seem to be a formidable one unless infection occurs only at an early stage of leaf development.

Information was also obtained on line leaves from Zanzibar. The very young leaves are susceptible to a *Gloeosporium*, but as they grow older they harden and lose susceptibility. The results of analysis are shown in Table II.

When losing susceptibility, the leaves were three-quarters fully grown. At this stage waxy materials were almost fully formed but the cutin was only one-third of that in the mature leaves. If the cutin in the cuticle were the only factor involved in the loss of susceptibility, a level of 50 $\mu\text{g./cm}^2$ or more would seem to be inimical to the fungus.

Burchill (2) showed that primary infection of the apple by powdery mildew occurred in the leaf buds. Leaves of Bowden's Seedling (susceptible) and of Worcester Pearmain (relatively immune) were therefore examined at the

earliest possible stages to find whether the degree of susceptibility could be related to the levels of cuticle components. The results are given in Table III.

TABLE II.
CUTICLE COMPONENTS OF LIME LEAVES.
($\mu\text{g./cm}^2$).

	<i>Young, susceptible</i>	<i>Young, losing susceptibility</i>	<i>Old, non-susceptible</i>
Waxes : Surface and absorbed ..	17	84	94
Cutin : Upper surface ..	24	96	240
Lower surface ..		54	156

TABLE III.
CUTICLE COMPONENTS OF YOUNG APPLE LEAVES.
($\mu\text{g./cm}^2$).

	<i>Flower spur leaves</i>		<i>Vegetative leaves, just uncurled</i>	
	Bowden	Worcester	Bowden	Worcester
Waxes : Surface	44	23	57	50
Absorbed	30	45	27	29
Cutin (mean for both surfaces) ..	38	34	48	49

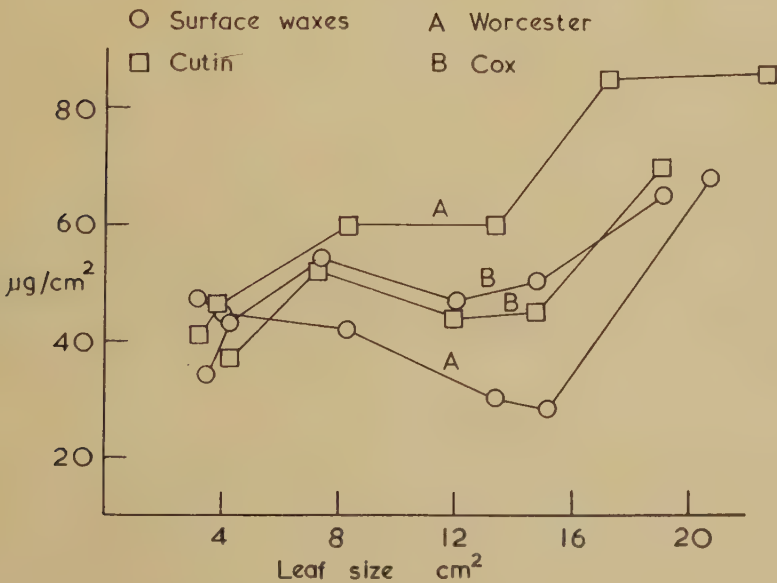


Fig. 2. Surface waxes and cutin during growth of apple leaves.

No differences were found at these stages that could account for the varying susceptibilities of the leaves to mildew.

Changes in surface waxes and cutin on Worcester Pearmain and Cox leaves were also followed during the period of leaf expansion. The results are shown in Fig. 2.

No differences were found between the small leaves of the two varieties, but the cutin in the fully expanded Worcester leaves tended to be higher than that in the Cox leaves. During the growth of the leaves, the formation of waxes and cutin hardly kept pace with leaf surface expansion.

The Effect of the Fungus upon Cuticle Components.

Comparisons were made between leaves showing disease and others of comparable size not visibly affected to find whether infection caused changes in the levels of cuticle components. The results of the examination of apple and turnip leaves are shown in Table IV.

TABLE IV.

CUTICLE COMPONENTS OF APPARENTLY HEALTHY AND DISEASED APPLE AND TURNIP LEAVES.
($\mu\text{g./cm}^2$).

	<i>Apple, Bowden</i>		<i>Turnip</i>	
	Healthy	Mildewed	Healthy	Mildewed
Surface waxes : wax	15	54	2	8
acids	6	29	nil	4
oil	9	10	1	2
Absorbed waxes	16	52	11	19
Cutin (mean for both surfaces) ..	68	24	14	7

TABLE V.

CUTICLE COMPONENTS OF APPARENTLY HEALTHY AND DISEASED MALUS AND CIDER APPLE LEAVES.
($\mu\text{g./cm}^2$).

	<i>Malus</i>		<i>Cider apple</i>	
	Healthy	Scabbed	Healthy	Mottled
Surface waxes : wax	14	16	21	20
acids	5	9	4	4
oil	24	25	18	17
Absorbed waxes	48	85	44	48
Cutin : upper surface	159	89	154	145
lower surface	120	122	115	103

The mildewed leaves showed higher waxy deposits than the symptomless leaves, and their cutin was considerably lower. Examinations were then made of apparently healthy and visibly scabbed leaves of an ornamental *Malus* and of normal and virus-mottled leaves of a cider apple variety. The results are given in Table V.

Scab infection was visible only on the upper surface of the affected *Malus* leaves and there the cutin was markedly depressed; the cutin on the unmarked lower surface was unchanged. In the cuticle components the virus-mottled apple leaves showed no differences from the symptomless leaves.

Comparisons were also made between apparently healthy and mildewed Virgo rose leaves and those of the resistant variety Peace. Before the analysis of the mildewed Virgo leaves, the fungus was gently removed by brushing, leaving clean surfaces. On extraction separately with chloroform, the fungal mass yielded waxy material equivalent to about 7 $\mu\text{g./cm}^2$ of leaf surface. The results are shown in Table VI.

TABLE VI.
CUTICLE COMPONENTS OF APPARENTLY HEALTHY AND DISEASED ROSE LEAVES.
($\mu\text{g./cm}^2$).

			<i>Virgo</i>		<i>Peace</i>
			Healthy	Mildewed	Healthy
Surface waxes :	wax		19	22	25
	acids		8	10	6
	oil		3	4	4
Absorbed waxes :	wax		33	12	30
	acids		19	23	11
	oil		91	68	58
Cutin (mean for both surfaces)		..	101	25	77

The mildewed Virgo leaves showed slightly larger deposits of surface waxy materials than the symptomless leaves, the excess being about equal to the amount of waxy substance recovered from the fungus. The cutin in the mildewed Virgo leaves was one-quarter of that in the apparently healthy leaves, but no differences were found between the cuticle components of the apparently healthy Virgo and Peace leaves that could be related to the difference in susceptibility of the two varieties.

The Effect of Cuticle Components upon the Fungus.

The cuticle fractions, as isolated, were examined for their effect upon mildew spores. Difficulties arose in obtaining a satisfactory test method. The powdery mildews cannot be grown upon artificial media and spores collected from the field show poor germination. A spore suspension method was inapplicable because the cuticle components are water-repellent; tests were therefore made of the reactions of spores obtained from natural infections and dusted on

to films of the materials (about $30 \mu\text{g./cm}^2$) deposited on glass slides. Germination counts (five each of 200 spores) were made after 24 hr. in the dark at 20°C . The test procedure had the advantage of simulating, to some extent, the conditions on the surface of the leaf.

Bioassays using apple and rose mildews could not be completed before supplies of suitable spores failed; examinations were therefore made with strawberry mildew spores which were amply yielded over a long period. The results of tests of the rose cuticle fractions are shown in Table VII.

TABLE VII.

EFFECT OF ROSE CUTICLE COMPONENTS UPON SPORES OF STRAWBERRY MILDEW.
(% germination).

				Healthy	<i>Virgo</i> Mildewed	<i>Peace</i> Healthy
Surface waxes :	wax	..	..	60	17	46
	acids	..	..	58	12	48
	oil	..	..	74	5	35
Absorbed waxes :	wax	..	..	6	5	11
	acids	..	..	27	17	21
	oil	..	..	12	5	10
Cutin acids		..	..	4	1	6

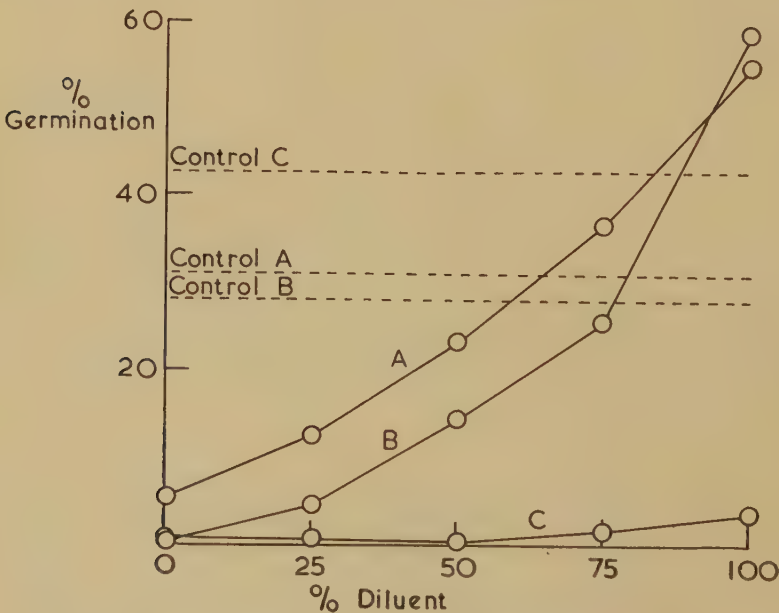


Fig. 3. A : Surface waxes of *Virgo* mildewed leaves diluted with surface waxes of *Peace* leaves ; B : Cutin acids of *Virgo* mildewed leaves diluted with surface waxes of *Virgo* healthy leaves ; C : Cutin acids of *Virgo* mildewed leaves diluted with cutin acids of *Peace* leaves.

Control counts on glass slides gave germination percentages ranging from 33 to 44; spores on comparable films of stearic acid germinated normally. The surface wax fractions of the apparently healthy Virgo leaves stimulated germination, the absorbed wax fractions of all leaves tended to suppress germination and a marked fungitoxic effect was shown by the cutin acids. The surface wax fractions of the mildewed Virgo leaves unexpectedly reduced germination.

These effects were confirmed in tests in which germination-inhibiting components were diluted with increasing amounts of stimulating fractions (Fig. 3, A and B). Mixtures of cutin acids were also used (Fig. 3, C).

The suppressive action of the cutin acids was lost when they were diluted with three times their weight of surface waxes. The effect of the cutin acids was not specific; those of apple leaves, for example, reduced the germination of spores of strawberry and rose mildews.

Discussion.

The degree of resistance of apple and rose leaves to their mildews does not seem to be related to the levels of deposition of waxes or cutin. Leaves of susceptible and of resistant apple varieties, taken at an early stage in development, showed no differences in these cuticle components; differences may occur, however, in the cuticles of the leaves in bud at the time infection occurs, although this possibility is not considered likely. The young lime leaves, carrying little cutin, were susceptible to *Gloeosporium* but the hardened leaves, bearing appreciable deposits of cutin, no longer showed the disease. On the other hand, the *Euonymus* leaves, infected by mildew presumably through a considerable layer of cutin, continued to support the disease despite large increases in cutin formation. The falling-off in infectivity with age of the lime leaves indicates a relationship between susceptibility and cutin deposition, but some other resistance factor, perhaps nutritional, may become operative in the lime but not in the *Euonymus* leaves as they mature.

The cutin is regarded as the toughening component of the leaf cuticle, but is it necessarily tough to a fungus? Cutin absorbs water, which swells the spongy framework and maybe permits easier invasion; this may account for the greater incidence of some diseases under humid conditions. The physical nature of the cutin and its degree of dilution with other materials may also be important. Roberts *et al* (3) observed that the cutin of the McIntosh apple leaf occurred in the form of lamellae and was traversed by channels of pectinaceous material. Wax canals and linked pockets of wax may also be present, and such channels could be utilized by the infection thread when negotiating the cutin layer. The cutin may be diluted with sugars and other materials; we have found that wax-free apple fruit cuticles yield varying amounts of methanol- and oxalate-soluble substances, and this may be true of leaf cuticles. The sugar contents of the leaf cuticles of varieties of differing susceptibilities to powdery mildews are of interest in view of the report that these are "high sugar" pathogens (4). Susceptibility may be influenced by the ability of the surface layers to support the mildew whatever the level of cutin.

Scabbed and mildewed leaves showed lower levels of cutin than apparently healthy ones. Badly diseased leaves are dry and brittle, probably because of loss of water through the weakened cuticles. Of the isolated rose cuticle

fractions, the cutin acids showed the greatest inhibitory effect on mildew spores. The surface waxes of the apparently healthy leaves increased and those of the mildewed leaves reduced germination. The inhibitory action of the waxes from the diseased leaves is difficult to understand but may be an expression of the plant's reaction to infection; occasional reports have been made of the production by plants of fungus-suppressing agents in response to invasion (see for example (5)). Whether the cutin acids play any part in protecting, or freeing a leaf from infection is not known; at present there is no evidence that they are liberated in an active form in the degradation of cutin by the fungus, neither is there any assurance that they are not changed in the process of chemical isolation. If liberated in the cuticle in the form in which they are separated chemically, any inhibitory action is likely to be reduced by dilution of the acids with other cuticle components. The factors underlying resistance are obviously complex. It seems unlikely that the waxes or cutin exert a controlling influence or that a thickened cuticle necessarily imposes a barrier to infection. More information is needed on the characteristics of cuticles, especially on the relative proportions of cutin and other materials, at the time invasions occur.

Summary.

Investigations are reported on the levels of waxes and cutin in leaves susceptible and resistant to fungi. No differences were found in these cuticle components that could be related to the varying susceptibilities of apple and rose varieties to mildews. Lower levels of cutin were found in scabbed and mildewed leaves than in apparently healthy leaves. Cutin acids derived chemically from cutin suppressed the germination of mildew spores. The composition of the cuticle in relation to invasion is discussed.

Acknowledgments.

We are indebted to Mr. R. F. Batt for the analyses of apple leaves during growth and to Dr. B. E. J. Wheeler of the Department of Agriculture, Zanzibar, for the supplies of lime leaves.

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STUDIES ON PLANT CUTICLE: V. THE CUTICLE OF APPLE FRUITS IN RELATION TO DAMAGE BY MERCURY.

By R. F. BATT and J. T. MARTIN.

Apple varieties differ considerably in their susceptibility to organo-mercury fungicides. Bramley trees may be sprayed with impunity, Worcester Pearmain trees are often treated without ill-effect, but Cox's Orange Pippin are easily damaged, especially under wet conditions. Affected fruits show localized discoloured areas on the skin which extend into the flesh. The mercurials must presumably penetrate the skin in the areas showing a toxic effect; an investigation was therefore begun in 1960 to find whether susceptibility to damage could be related to the composition of the fruit cuticle.

Experimental.

Fruits were taken late in July from the Bramley trees sprayed from shortly after bud-burst with phenylmercuric acetate, with addition of a wetter, in the experiment described on p.86, from Worcester Pearmain trees subjected to the same treatments as the Bramley trees, and from Cox trees sprayed only in mid-June and three weeks later. Defoliation of the Cox trees was evident at the time of the second application and few fruits remained on the trees when the samples were taken; the Worcester and Bramley trees and fruits were unaffected.

The cuticles of the fruits were examined by the method described elsewhere (1). The fruits were weighed, surface areas were determined from mean diameters, and surface waxes were removed by immersion in four successive portions of chloroform at room temperature. One hundred discs of peel, each 1cm.², were then taken and cellular material was scraped away as cleanly as possible, after soaking in water, without damaging the cuticular membranes. Sections showed that some flesh remained attached. The membranes were dried to constant weight, Soxhlet extracted successively with chloroform and methanol, and refluxed in turn with ammonium oxalate-oxalic acid solution, 1.25 per cent w/v sulphuric acid and 0.2 per cent sodium hydroxide solution. Cutin was then determined by saponification with 3 per cent alcoholic potassium hydroxide solution.

The chloroform and methanol extractions were designed to remove occluded waxes, pigments and sugars, the oxalate solution to remove pectinaceous material and the dilute acid and alkali to clean up the membranes before saponification. The membranes were washed and dried to constant weight after the methanol, oxalate and sodium hydroxide treatments, to determine the proportions removed at each stage.

Results.

Details of the samples taken are shown in Table 1.

TABLE I.
FRUITS TAKEN FOR ANALYSIS.

	Cox		Worcester		Bramley	
	Sprayed	Unsprayed	Unsprayed	Sprayed	Sprayed	Unsprayed
Date	22 July	12 Aug.	13 July	28 July	20 July	12 Aug.
No. of fruits	32	15	36	13	17	11
Average wt, g.	16.1	43.3	19.6	48.4	42.1	63.4
Average surface area, cm ² .	27	52	35	58	50	64

Surface waxes

The amounts of waxy materials removed by successive immersions in chloroform are shown in Table II.

TABLE II.
REMOVAL OF SURFACE WAXES.
(mg./cm².)

	Cox		Worcester		Bramley	
	Sprayed	Unsprayed	Unsprayed	Sprayed	Sprayed	Unsprayed
Immersion 1	0.34	0.36	0.27	0.30	0.38	0.48
2	0.10	0.10	0.08	0.09	0.11	0.13
3	0.07	0.07	0.06	0.07	0.07	0.08
4	0.05	0.06	0.04	0.05	0.05	0.07
Total	0.56	0.59	0.45	0.51	0.61	0.76

60 per cent of the total waxy substances recovered was obtained from the first immersions. The removal of surface waxes from fruits with chloroform was not so clean-cut as that from leaves (see p.103), but further immersions were not made because of the risk of extracting occluded waxes. The amounts obtained from the four immersions were taken to represent the waxy coverings on the fruit surfaces.

Fractionation of the cuticular membranes

The results obtained when the surface wax-free membranes were progressively extracted with solvents are shown in Table III.

TABLE III.
FRACTIONATION OF CUTICULAR MEMBRANES.
(mg./cm².)

	Cox		Worcester		Bramley	
	Sprayed	Unsprayed	Unsprayed	Sprayed	Sprayed	Unsprayed
Original average wt.	3.76	2.65	4.34	2.56	3.61	2.33
Removed by chloroform and methanol	0.09	0.20	0.25	0.35	0.69	0.43
Removed by oxalate	2.52	1.17	2.80	0.84	1.41	0.27
Removed by acid and alkali	0.91	0.64	0.51	0.60	0.66	0.58
Cutin	0.18	0.52	0.76	0.70	0.80	1.02

The weights of the cuticles per unit area of surface, obtained by the addition of the values for surface waxes to the weights of the membranes, are shown in Table IV.

TABLE IV.
WEIGHTS OF CUTICLES OF APPLE FRUITS.
(mg./cm².)

	Cox		Worcester		Bramley	
	Sprayed	Unsprayed	Unsprayed	Sprayed	Sprayed	Unsprayed
Surface waxes plus cuticular membranes	4.32	3.24	4.79	3.07	4.22	3.09

Between the dates of sampling each variety, the weight of cuticle per unit area decreased by about 30 per cent, the formation of new cuticular material failing to keep pace with the extension of the surfaces. During these periods, changes occurred in the compositions of the membranes below the surface waxes as shown by the percentages of materials extracted by the different solvents (Table V).

TABLE V.
COMPOSITION OF CUTICULAR MEMBRANES
(per cent)

	Cox		Worcester		Bramley	
	Sprayed	Unsprayed	Unsprayed	Sprayed	Sprayed	Unsprayed
Removed by chloroform and methanol	2	8	6	14	19	18
Removed by oxalate	67	44	64	33	39	12
Removed by acid and alkali	24	24	12	23	18	25
Cutin	5	20	18	27	22	44

The membranes contained appreciable amounts of materials extractable by oxalate solution, but the proportions of these fell during the intervals between sampling. Concurrently, the percentages of cutin in the membranes increased.

Discussion.

The separation of the flesh from the peel discs was assisted by soaking them in water, and this procedure may have removed water-soluble substances from the cuticular membranes. The values obtained for the materials extracted from the membranes by the solvents included the unknown contributions made by the attached cellular material; these, however, were probably small and all the samples received the same treatment. The attached flesh was lost in the oxalate extraction; the residues after saponification, consisting of cellulosic material from within the cutin layers, on no occasion exceeded 4 per cent of the original membranes.

Tentative suggestions may be made to explain the different susceptibilities of the three varieties. Damage to susceptible varieties occurs where large drops of spray fluid hang from the fruits. As the drops dry, the mercurial becomes concentrated over small areas. Under non-humid atmospheric conditions, the cuticles are contracted, the drops dry quickly and penetration is probably limited. Under wet conditions, the drops dry slowly and the cuticles are in a swollen receptive state; then penetration is aided, the mercury builds up in a localized zone and damage results.

Surface waxes may be expected to present the first barrier to penetration, but differences in susceptibility cannot be related to differences in wax deposition; the susceptible Cox fruits carried at least as much surface waxes per unit area as the Worcester fruits (Table II). Neither does susceptibility seem to be due to a light membrane below the surface waxes; the original weights, per unit area, of the surface wax-free membranes of the Cox fruits were equal to those of the other varieties (Table III). Differences were seen, however, in the cutin. The membranes of the Cox fruits contained lower levels of cutin than those of the Worcester fruits of comparable size, and those of Bramley contained more cutin than those of Worcester (Tables III and V). A heavy membrane is therefore not necessarily a barrier to penetration; the operative factor seems to be its content of cutin. The more the cutin is diluted with pectinaceous and other materials, the greater is the ease of penetration of the mercury. As in the work reported on p102, the results draw attention to the probable importance of the non-cutin components of the cuticle.

The outstanding result was the very low content of cutin (0.2 mg./cm^2 .) in the membranes of the mercury-treated Cox fruits taken on the 22nd July. Other work (2) on the development of the cuticles of apple fruits during growth has shown that untreated Cox fruits of comparable size would be expected to show a level of cutin not less than 0.5 mg./cm^2 . The development of cutin is probably an enzymatic process which may be impaired by the mercury. The possibility therefore arises that the susceptibility of Cox fruits to mercury is due not only to a naturally low content of cutin in their cuticular membranes which permits penetration, but also to the suppression by the mercury of further cutin formation as the fruits grow. This possibility is under examination.

Summary.

Work is reported on the composition of the cuticles of Cox's Orange Pippin, Worcester Pearmain and Bramley apple fruits in relation to spray damage. Susceptibility to damage was not related to the levels of deposition of surface waxes or of the cuticular membranes below the surface waxes, but seemed to depend upon the content of cutin in the membranes. The possibility that mercury suppresses the formation of cutin is discussed.

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BLACK CURRANT LEAF SPOT : OBSERVATIONS ON VARIETAL SUSCEPTIBILITY.

By A. T. K. CORKE and D. WILSON.

Early work at Long Ashton showed that certain manuring and pruning treatments increased the vigour of black currant bushes, rendering them less susceptible to infection by leaf spot, *Pseudopeziza ribis*. Other methods examined for reducing the amount of disease during the summer months were spraying, and the raising of new varieties that would have, among other desirable qualities, some degree of resistance to infection. While it is impracticable to keep infection at a low level by manuring and pruning, spraying with copper after picking has been shown by Marsh and Maynard to increase the weight of the buds formed soon afterwards by 15%, and the crop obtained in the following year by 26% (3). Others, using copper before and after picking, have found fruit size and quality improved, and the crop weight more than doubled in the following year (2). Although reductions in infection of this order may not result from crosses of varieties showing only small differences in susceptibility, it is clearly advantageous to include increased resistance to leaf spot as one of the objects of any black currant breeding programme.

In 1953, when over 2 acres of six black currant varieties were planted, an opportunity was presented for studying their relative susceptibilities in the absence of sprays for the control of leaf spot. Although these varieties were not specially selected for the purpose of comparing susceptibility to leaf spot, they were of particular interest because three of them were raised at Long Ashton; all but one are widely grown commercially.

Experimental.

In the winter of 1953, $2\frac{1}{4}$ acres were planted with black currant bushes at distances of 3 ft. $\times$ 8 ft., with two 12 ft. alleyways running the full length of the plantation. Two-year-old bushes of four varieties, Cotswold Cross, Baldwin, Westwick Choice and PS/24/1922 (a Long Ashton seedling of Boskoop origin)

were planted in thirty-two 2-row plots statistically arranged : two rows of the variety Mendip Cross were planted as buffers between the four main varieties on half the area, and Wellington XXX as buffers on the other half.

No sprays for the control of leaf spot were applied. Assessments of the amount of leaf spot were made annually from 1957 to 1960 using the method of comparison with percentage-infection diagrams already described (1). By the summer of 1957, infection was sufficiently well distributed on all varieties for three assessments to be made before leaf-fall ; in subsequent years assessments were made every two weeks from the first appearance of spots.

Results.

In 1957 the most heavily infected variety was Westwick Choice, while Baldwin was slightly less affected : on Cotswold Cross, which was one of the least infected in July, the disease subsequently developed more quickly than on any other variety, with the result that by October it was as heavily infected as Baldwin. In 1958 Cotswold Cross was the most heavily infected, Westwick Choice and Baldwin becoming infected to the same extent as Cotswold Cross only towards the end of the summer. Subsequently, Baldwin was the variety most subject to infection, although Cotswold Cross and Westwick Choice were almost as severely affected. The lowest level of infection during the 4 years was on Mendip Cross and PS/24/1922, the latter being the less susceptible.

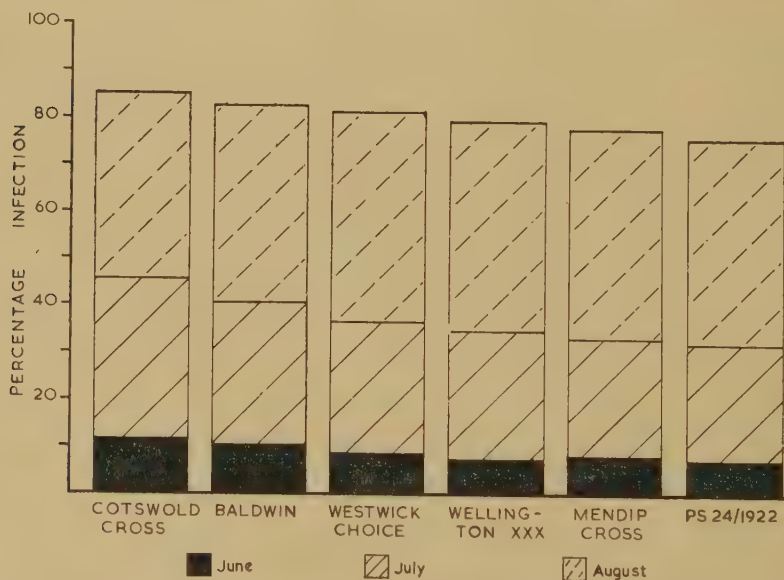


Fig. 1. Mean percentage infection on six black currant varieties in a mixed plantation ; 1958-60

The mean percentage infection recorded in each month during the last 3 years is shown diagrammatically in Fig. 1. It can be seen that up to the end of August the rate at which leaf spot developed on Cotswold Cross was higher than that on any other variety. As a result of a lower rate of increase on this variety during September, the others eventually became equally infected.

Since each plot of the four main varieties consisted of two rows of bushes with a row of a different variety on either side, each row might have been influenced to a different degree by the amount of infection on the next row. In order to determine whether a variety such as PS/24/1922, with a low level of infection, was more heavily infected when next to one with a higher level of infection, such as Cotswold Cross, it was necessary to re-examine the records of percentage infection. The mean percentage infection recorded during the 4 years on each row of each variety is given in Table I in relation to the adjacent variety. Where a variety was on the outside of the plantation, it is shown as adjacent to itself.

TABLE I.

THE EFFECT ON MEAN PERCENTAGE INFECTION (1957-60) OF PROXIMITY TO VARIETIES OF DIFFERENT SUSCEPTIBILITIES.

Variety assessed	Adjacent Variety						Mean
	Cotswold Cross	Baldwin	Westwick Choice	Wellington XXX	Mendip Cross	PS/24/1922	
Cotswold Cross ..	54.8 *(2)	63.2 (1)	59.6 (2)	56.9 (4)	62.4 (4)	60.0 (3)	59.5
Baldwin	61.2 (1)	51.8 (2)	54.5 (2)	56.5 (4)	56.7 (4)	59.8 (3)	56.8
Westwick Choice ..	56.6 (2)	54.3 (2)	53.6 (4)	56.5 (4)	54.8 (4)	—	55.2
Wellington XXX	50.9 (4)	53.4 (4)	54.0 (4)	—	—	52.3 (4)	52.7
Mendip Cross ..	51.7 (4)	51.9 (4)	50.0 (4)	—	—	50.0 (4)	50.9
PS.24/1922 ..	50.5 (3)	51.7 (3)	—	48.5 (4)	52.2 (4)	44.8 (2)	49.5
Mean	54.3	54.4	54.3	54.6	56.5	53.4	

*Figures in brackets are the total number of rows next to a given variety.

The lowest figure for each variety, given in Table I, occurs where the rows were on the outside of the plantation; both partial exposure and the absence of other varieties may have helped to reduce infection on these rows. Although the varieties are arranged vertically and horizontally in the order of susceptibility shown in Figure 1, there is little difference between the means at the base of each column. As these figures should give some general indication of the influence exerted by each variety on every other one, it appears that no such influence exists.

In Fig. 2, the percentage infection on Cotswold Cross and PS/24/1922 is shown for the period 18th June to 10th September, 1958. Comparison of the rates of development of leaf spot on these two varieties shows that the infection differed by almost 25 per cent during that year, and suggests that some restriction on lesion development or sporulation occurred on PS/24/1922. It may be

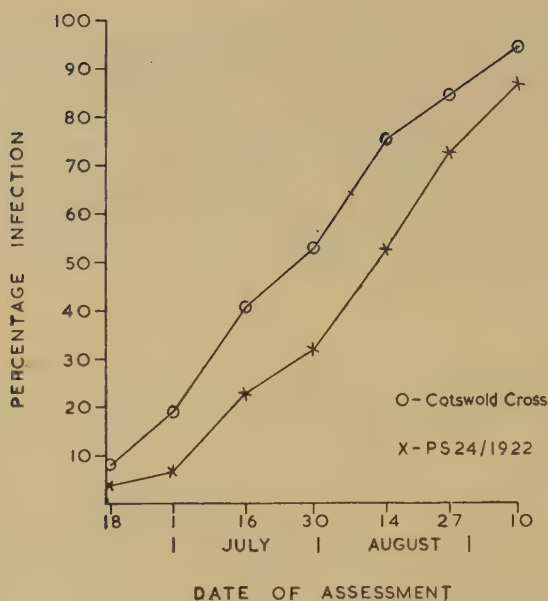


Fig. 2. Percentage infection of Cotswold Cross and PS/24/1922 ; 1958.

noted here that a difference of 10 per cent in the mean percentage infection of these varieties (Table I), does not fully reflect the much greater differences that were clearly visible in the field during each season.

Discussion.

It has been suggested above that partial exposure, or the absence of other varieties, may have helped to reduce the level of infection recorded on the outside of the plantation. Within a plantation, it might be expected that rain-splashed spores would carry infection from row to row with much the same facility as from bush to bush within a row, particularly in the late summer, when most of the seasonal growth has taken place. If the difference in the rates of development of this disease on Cotswold Cross and PS/24/1922, as shown in Fig. 2, is brought about by restricting development on the less susceptible variety, it follows that in isolation the disease would develop at a rate depending solely on the factors involved in the host/parasite relationship on the particular variety. In a mixed plantation, on the other hand, inoculum on bushes of PS/24/1922 might be expected to be increased when close to Cotswold Cross, with a consequent increase in infection : a similar effect in the reverse direction would not be expected. Although it appeared, from the results given in Table I, that no influence had been exerted by one variety on another of different susceptibility, it seems probable that the level of infection on each variety was raised by the presence of a more susceptible variety nearby. Differences in susceptibility would thus tend to be less marked than they would have been if each variety had been grown separately. Since there was less leaf spot on the outside rows of even the most susceptible variety, the effect must be attributed to partial exposure and the rapid drying of the leaves after rain, rather than to the absence of other more susceptible varieties.

The above observations show that differences in susceptibility to infection exist that are large enough to be measured by a method primarily developed for assessing the results of spray trials. Whereas varieties have previously been said to be more or less susceptible to leaf spot than Baldwin, for example, it should now be possible to determine the amount by which one variety differs from another in its susceptibility. Since it has also been shown here that the difference in infection between two varieties may be almost 25 per cent during mid-season (at about the time of flower initiation), the rate at which leaf spot develops may well have a much greater effect on the bush than has the final level of infection.

It is of interest to note that the three varieties least infected by leaf spot include Boskoop in their parentage. Baldwin, one of the most susceptible varieties, has been one parent of most recently-introduced varieties. A newly-published list of varieties of major commercial importance (4) names Baldwin and three others, each of which has Baldwin as one parent : four of the five varieties of minor importance named also have Baldwin ancestry. This emphasis on Baldwin as a parent in breeding programmes, while undoubtedly valuable for other characteristics, may well be responsible for the apparent lack of progress in obtaining varieties less susceptible to leaf spot. Experiments now in progress may show that lowered susceptibility can be obtained by using unfamiliar varieties of foreign origin as parents. A variety trial at Long Ashton has indicated that Silvergeiter is less infected than most English varieties ; many Russian reports refer to varieties resistant to leaf spot, and while this may be due to strain differences or weather conditions, these and other varieties would be worth trial under our own conditions before intervarietal crossing is discounted as a method for reducing susceptibility to this disease.

Summary.

Assessments of the amount of leaf spot on an unsprayed plantation of mixed black currant bushes (1957-60) showed that each of the six varieties had a different level of susceptibility to leaf spot : it seemed likely that in plantations of single varieties the differences would be greater. The value of such measurements in the breeding of new varieties is discussed.

Acknowledgments.

Grateful thanks are due to Mr. G. M. Clarke and the staff of the Statistics Section for the statistical analyses on which the results given here are based, and to the staffs of the Pomology and Plant Pathology sections for assistance in field recording.

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POPULATION STUDIES OF THE BLACK CURRANT GALL MITE (*PHYTOPTUS RIBIS* NAL.).

By B. D. SMITH.

A number of attempts have been made to measure bud populations of the Black Currant Gall Mite; recently Collingwood and Brock (1) have estimated the number of mites in a sample consisting of two groups of 5 galled buds collected at approximately fortnightly intervals over a two-year cycle. Since for the present investigation it was required to know the numbers of mites and eggs within buds, and the relative importance of various methods of dispersal, it was necessary to devise methods of sampling free-living populations.

Experimental.

Techniques for estimating numbers of mites and eggs within buds.

Most estimates of the intensity of infestation by the gall mite have been based on a visual examination of the buds. It has been found rare for two observers to agree as to the precise levels of infestation in identical samples: it is common to find mites in buds of normal size which are, however, more rounded than healthy buds; some workers classify these as healthy, other workers as infested. For simple control trials in which the same observer counts buds on treated and untreated bushes, visual estimates are adequate, but for comparing populations in different years, or for comparing estimates made by different workers, a technique less susceptible to bias is needed.

Hand dissection of buds has proved useful when only a few buds are being examined but much too tedious when the examination of large numbers of buds is necessary, as in the sampling of a plantation. The technique found most satisfactory has been to macerate a known volume of buds and to count the number of mites in samples of the macerate taken after sieving-out most of the plant material.

For each estimation, a number of buds just sufficient to fill a 2×1 " specimen tube are placed in a macerating machine (M.S.E. Homogeniser) with sufficient water (50 ml.) to bring the buds into the plane of the cutting unit. Maceration is continued for 1 minute with the machine at half speed (7,000 r.p.m.); this has been found to separate the maximum number of mites and to cause them the minimum amount of damage. The macerate is then poured on a brass wire sieve (900 holes per square inch) set in a filter funnel. The residue is washed on the sieve with 200 ml. of water, previously used for rinsing the macerating vessel. The washings are collected in a beaker and the suspension is kept well stirred while from it drop samples are taken and placed singly on black filter paper with a small pipette. It has been found advisable to refill the pipette before each sample is placed on the paper because of the rapid settling of mites within the pipette. Counts of whole mites are made after a few minutes, when the water has been absorbed by the paper: the area covered by the mites can be conveniently observed in the entire field of a low-power binocular microscope. A cardboard mask, with a hole equal in area to the mites and plant debris, placed on the filter paper, helps counting, and fine cotton threads placed across the mask also help when large numbers of mites have to be counted. The macerate contains just enough debris to outline the area to be scanned without obscuring the mites. The mean count of mites in

12 drop samples gives a sufficiently accurate estimate of the number of mites in the original volume of bud material. The technique gives some estimate of the numbers of eggs present, but these are more difficult to see on the filter paper and many are retained in the macerated debris.

A disadvantage of the technique is that it does not distinguish between the natural mortality of mites in buds and mortality caused by maceration ; for simple comparative estimates of population, or for spraying experiments in which controls are used, this will present no difficulties. The technique can be used to estimate low populations of mites even when their distribution in a plantation is patchy. The sampling method generally used has been to collect the terminal bud and every fifth successive bud from one main shoot and its side branches, chosen at random from bushes also selected at random. Buds are collected from bushes evenly spaced over the plantation until the volume required for maceration is obtained, or multiples of this volume of such a size that the numbers of mites in parallel samples do not differ significantly. Fluctuations in the numbers of eggs in buds were also measured by collecting large samples of infested buds at intervals, bisecting them, and counting all the eggs visible under a low-power binocular microscope. Counts of mites in these sectioned buds provided a useful check on the population fluctuations determined by maceration.

To determine the rate of increase in the numbers of mites inside buds it was necessary to count at intervals the numbers of mites and eggs in the same bud. After dissection of buds, however, there could be no further counts made on those buds, and to overcome this difficulty attempts were made to observe mites and eggs inside buds by X-ray photographs. The instrument used had a fine focus and stepless kilovoltage control claimed capable of producing sharply defined high contrast images that could be photomicrographically enlarged. With experience it proved possible to distinguish mite tissue from plant tissue, but because the mites moved during the exposures, the pictures were blurred, and where more than a few mites were present it was not possible to count them. The effect, if any, of the X-rays on the subsequent behaviour of the mites was not studied.

Estimation of numbers of mites outside buds.

Microscopic examination could not be used for counting mites outside buds ; a mechanical method of sampling was therefore devised primarily to investigate the factors affecting the rate of emergence from buds. The apparatus consisted of a test tube containing about 1" of water through which air was sucked by a small pump driven by an electric motor ; a guard tube was used to prevent drops of liquid from reaching the pump. A suction tube made from 5 mm. bore glass tubing was fitted so that mites were carried below the surface of the water in the test tube. The whole apparatus was portable and since the collecting tube was connected to the pump by a long piece of rubber tubing it could be used in the field in any part of a black currant bush. When the collection of mites was completed, the contents of the tube were passed through black filter paper and the mites counted under a low-power microscope. Some of the results obtained with this apparatus have been given in a previous paper (2). By attaching a time switch to the electric motor it was possible to estimate populations at a given site at intervals throughout a 24 hour period.

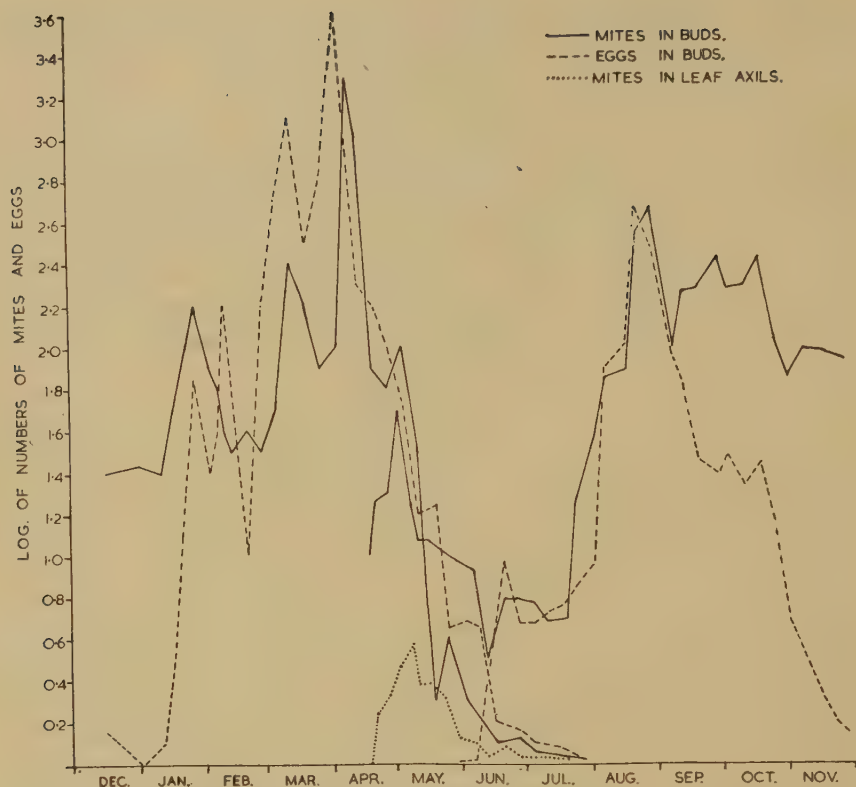


Fig. 1. Numbers of mites and eggs per bud and mites per leaf axil; Long Ashton, 1959-60.

Fluctuations in populations of mites and eggs.

Fig. 1 shows the fluctuations in populations of mites and eggs in a plantation at Long Ashton, from December, 1959, to December, 1960, expressed as the logarithm of the number of mites and eggs per bud and the logarithm of the number of mites per leaf axil. The population estimates were obtained by dissection and by the mechanical sampling technique. Similar fluctuations were observed in samples taken from the same plantation during 1958 and 1959.

During December the number of mites remained fairly constant and the number of eggs fell to zero. This is the time, as previously shown (3), when most mites appear to be in a diapause. In early January, egg laying began again, and the populations of both mites and eggs rose to a similar size by early February, falling again during this month. There was another marked increase in the numbers of mites and eggs in mid-March and another even more pronounced in early April, the egg peak exceeding that of the mites and preceding it by about one week. This peak in the first week of April corresponded with the 'grape' stage in the variety Baldwin at Long Ashton. Whilst mites may be observed emerging from infested buds in early March, the majority emerge from the 'grape' stage onwards, when the buds have swelled and opened sufficiently to release them, the most important factor affecting their rate of emergence being temperature (2).

It will be seen that, shortly after the 'grape' stage, mites were recorded both in the leaf axils of the developing shoots and also in the axillary buds themselves, whilst the numbers of those left in the old infested buds fell rapidly. There was considerable mortality amongst mites which had penetrated into new buds; this is shown by the fall from early May to early June. Eggs were first observed in the new buds at the end of May, approximately 6 weeks after the first observed entry into an axillary bud. Populations of eggs and mites rose to a peak in the third week of May and to a much higher peak in late August. From this time the egg and mite populations fell: the numbers of eggs fell almost continuously to December, whilst the mite population fluctuated around a not well-defined level in October before falling again. Mites and eggs were recorded in old infested buds until the end of July, even though these had been drying out from May onwards.

It has so far been impossible to culture the gall mite outside black currant buds and thus to obtain precise information on the mean generation times under various conditions. Kieffer (4) has stated that the period from egg-laying to the adult stage is 10 to 14 days for Eriophyids, and since an interval of several weeks elapses between the attainment of suitable feeding sites at the centres of newly formed axillary buds and the onset of egg laying, it is suggested that the mean generation time is at least 4 weeks. Field sampling has supported this suggestion and has indicated that there are at least six generations in a year: three between January and the 'grape' stage in April, and three between June and November. Reproduction continues, however, in the old infested buds after the major emergence from the 'grape' stage onwards, and the two small peaks observed at the beginning and end of May may well result from the production of two more generations. In Russia, Savzdarg (5) found that there were three generations in the summer and autumn, and only two in the spring.

Mortality factors.

The importance of desiccation as a mortality factor has been previously discussed (2); it is undoubtedly responsible for the death of a very large proportion of migrating mites. Starvation is also believed to be an important factor: mites that have taken longer to reach suitable feeding sites, at the centres of new axillary buds, appear to survive for a shorter time than those with shorter free-living periods; they have used more energy, either by their own movement or in attachment to transporting agencies, and this may well account for some of the mortality in the new buds. This is shown in Fig. 1 after the number of mites penetrating new buds has reached a peak.

Polyphagous predators, including some free-living mites, are commonly found inside old infested buds after the 'grape' stage, but they appear to be responsible for only a small amount of mortality, and use the buds, in part at least, for shelter. There are many polyphagous predators in black currant plantations that mites may encounter whilst migrating, but these have not been observed to kill more than a few mites, even at the height of migration. There is one predator, however, which appears to feed entirely on mites but again makes little impression on bud populations. This is a Hymenopteran larva, *Tetrastichus eriophyes* (Taylor), which spends more than half the year feeding on mites inside buds; in some plantations up to 30% of the infested buds have been observed to contain one or more of these larvae.

Of perhaps greater importance than these larvae are the small birds that peck swollen buds during the winter. It has been suggested that the birds are seeking the Hymenopteran larvae; they often destroy large numbers of buds in localized areas before changing their diet.

Summary.

1. Various techniques for estimating numbers of Black Currant Gall Mites and their eggs inside buds are discussed, and a bud maceration technique is described that gives a rapid and reliable estimate of the number of mites in the buds and a less accurate estimate of the number of eggs.
2. A suction apparatus to measure populations of mites after emergence is described.
3. Counts of mites and eggs inside buds, and of mites in leaf axils, are shown for a period of twelve months at Long Ashton. Besides major peaks there are several minor peaks that suggest that there are at least six generations of mites in one year.
4. Factors causing the death of mites are considered: predation is believed to be of little importance compared with desiccation and starvation.

Acknowledgments.

Thanks are due to Mr. E. A. Fribbins for his advice and help in constructing apparatus, to Mr. A. A. Baker for help in recording, and to Associated Electrical Industries Limited for the loan of X-ray apparatus.

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THE CONTROL OF THE BLACK CURRANT GALL MITE (*PHYTOPTUS RIBIS* NAL).

By B. D. SMITH.

For many years the most common method for the control of the Black Currant Gall Mite has been the application of lime sulphur at the 'grape' stage of plant development; the concentrations of lime sulphur have varied from 2% to 8% and have sometimes been even higher. In recent years attempts have been made to find new chemicals to control the mite, as concentrations of lime sulphur as low as 2% are known to cause leaf scorch and reduction of crop if applied under unfavourable conditions. Several chemicals, of which endrin is the most notable, have been shown to be very effective biologically, when sprayed on bushes from the 'grape' stage onwards, by virtue of their persistence; but it is this characteristic which has mainly limited their use in commercial plantations. In the present investigation, control has been attempted at three times of the year: prior to bud-burst, from the 'grape' stage onwards, and after fruit picking. Both foliar sprays and soil applications have been used.

Soil Applications.

It has been shown (1) that in the winter the mites have a diapause and that after this diapause has been terminated the host plant controls their rate of reproduction. Sampling has shown that there is a marked increase in egg production in January, and it is believed that the mites are actively feeding from this time onwards. There is some uptake of water by the plant even before the buds have opened, and it was thought that systemic acaricides or insecticides might reach the mites if applied to the soil before leaves appeared. By using radio-active phosphate it was, however, shown that once leaves had appeared the transpiration stream was directed past the infected buds, which are invariably without leaves, and was therefore unlikely to carry systemic materials to the sites at which the mites were feeding.

In February, 1959, products containing schradan, mevinphos, dimefox, demeton-methyl, dimethoate and fluoroacetamide were made up in water at the manufacturers' highest recommendations for aphid control, and also at double these strengths. They were watered round some bushes at the rate of $\frac{1}{2}$ gallon per bush and round others at 1 gallon per bush. For several days after the application of schradan, the mites inside buds on plants treated with this chemical were in a much more active state than those in the buds of untreated bushes or those treated with other chemicals. It has been shown (1) that slight interference with the physiology of the plant can upset the feeding of mites, causing them to move more actively and reduce their rate of egg laying. Counts of mites and eggs showed that with none of the chemicals used was there any significant increase in mortality.

The same materials were applied to the soil on several occasions in 1959 after the fruit had been picked, in an attempt to get some systemic material into the infested buds as the sap flow began to be reversed. Some mortality of mites was achieved with fluoroacetamide but not sufficient to suggest that the procedure would be useful as a control measure. Soil applications made just after the buds had opened, and before many mites had migrated, again showed fluoroacetamide to be the material with greatest effect, although this was insufficient to promise practical control.

Foliar Applications.

The same compounds used in soil applications were applied at the same concentrations to bushes as soon as the leaves began to appear, and also on the same occasions that soil applications were made after picking; they were applied to run-off using a knapsack sprayer. Fluoroacetamide once again was clearly the most effective but not sufficiently so to suggest further trials; at the higher concentrations some signs of phytotoxicity were observed.

Spraying experiments have therefore been subsequently confined to the conventional period: from the 'grape' stage onwards. Collingwood and Dicker (2) have described screening trials with a number of chemicals applied during this period; these have not yet revealed a chemical which can safely replace lime sulphur in commercial fruiting plantations whilst giving adequate control of the mite. For the complete eradication of mites it has been found necessary, during the present work, not only to kill all the mites already present in a plantation, but also those arriving from outside. Whilst the most effective sprays for killing mites inside, or emerging from, 'big buds' are those applied

at the 'early grape' and 'grape' stages, it is not possible with a single spray at this time, or even with a second one in the post-blossom period, to cover all the new growth as it appears and thus protect the new axillary buds from mites which have either escaped these sprays or have immigrated.

Attempts were made to give as complete a cover as possible with an acaricide to the new growth from the 'early grape' stage almost until picking time. When lime sulphur with a succinate wetter was sprayed by hand lance at a concentration of 1% at fortnightly intervals, complete control of the gall mite was achieved. However, such a spray programme is commercially impracticable for many reasons, amongst which are the increasing difficulty of penetrating plantations as the season progresses, the large volumes of spray required to ensure complete cover, and the possibility of crop yield being reduced by leaf scorch. By reducing the number of applications and the volume of spray to less than $\frac{1}{2}$ gallon per bush it was hoped to minimize these difficulties without reducing the biological efficiency of the lime sulphur. Ahlberg (3), who used lime sulphur in Sweden at weekly intervals, states that it is the number of spray applications, rather than the concentration, which is critical. Practical control is obtained in plantations in the Netherlands with 4 or 5 applications of acaricides at weekly intervals from the beginning of mite migration. Trials at Long Ashton in 1959 and 1960 with 1% lime sulphur showed that a two-weekly interval between applications was almost as effective as a weekly interval and very much more effective than intervals of three and four weeks.

To obtain information on the minimum number of applications necessary to obtain practical control of the gall mite at Long Ashton, blocks of 14 bushes of the variety Baldwin were in 1960 given up to six cumulative treatments with 1% lime sulphur, sprayed by hand lance to 'run-off' at fortnightly intervals from the 'grape' stage onwards; one block of bushes was left unsprayed. The effectiveness of the treatments was assessed by counting both galled and apparently mite-free buds on each bush in February and again in November: this permitted the variation within the block to be calculated.

The results of these applications are given in Table I. It will be seen that in untreated bushes there was an average rise in infestation of 233%. Whereas a single application reduced the infestation by 4.5%, a second gave 61.8% reduction and a third 81.4% reduction; in fact with three treatments there was 100% control on two bushes. No very large average reduction was caused by the fourth to sixth applications but it will be seen that they reduced the variation between bushes as shown by the approach of the minimum percentage reduction to the maximum.

The results of a similar experiment to find the relative efficiency of $\frac{1}{2}$ % lime sulphur are also shown in Table I: this experiment was carried out on Mendip Cross, whose growth stages during the period of the experiment closely approximated to those of Baldwin. With $\frac{1}{2}$ % lime sulphur the effectiveness of the 'grape' stage application appears greater than that of 1% lime sulphur on the variety Baldwin; this is most probably because the infested buds of Mendip Cross were much larger than those of Baldwin and thus opened and allowed mites to migrate earlier. As with 1% lime sulphur, considerable increases in effect resulted from the second and third applications but little or no improvement was made by the next three applications.

Complete control was achieved in one bush of Mendip Cross after only

TABLE I.

EFFECTS OF LIME SULPHUR SPRAYS ON INFESTATIONS OF GALL MITE IN BLACK CURRANTS.

Number of applications	Average % buds infested per block		% Reduction or increase		
	Pre-treatment	Post-treatment	Average	Max.	Min.
<i>Lime Sulphur 1% ; fortnightly applications. Variety Baldwin.</i>					
0	8.96	29.7	+233	+30.0	+533.3
1	8.86	8.4	4.5	65.7	+37.4
2	7.69	2.9	61.8	85.0	44.4
3	8.17	1.5	81.4	100.0	41.9
4	7.08	0.6	91.4	100.0	71.1
5	7.35	0.6	91.7	100.0	78.7
6	10.88	0.5	96.8	100.0	90.2
<i>Lime Sulphur ½% ; fortnightly applications. Variety Mendip Cross.</i>					
0	6.78	13.28	+97.0	5.4	+105.8
1	6.58	3.23	50.7	87.6	27.0
2	7.46	1.35	82.4	100.0	63.0
3	5.53	0.22	96.3	100.0	88.7
4	4.74	0.39	91.4	100.0	71.4
5	4.96	0.06	97.9	100.0	91.6
6	5.72	0.11	98.2	100.0	90.3

two applications, and after three applications the figure for the minimum effectiveness of the treatment had closely approached that for the maximum. In the 1960 season ½% lime sulphur therefore appeared at least as effective as 1%.

In other experiments, individual bushes with bud infestations of up to 60%, much higher than those likely to be met in typical commercial plantations, were given cumulative 1% lime sulphur applications ; infestation was reduced to almost as low a level as in the trials described above. Most of the trials have been carried out on bushes with levels of infestation between 5% and 10%, commonly met with in commercial plantations. A grower should consider the presence of 5% big buds as a severe infestation and take immediate control measures.

Optimum Time of Application.

When different mite-free plants were exposed at weekly intervals to the same infested material during 1959, the highest percentage infestation of new buds occurred in the variety Baldwin just before full blossom (4). In a similar experiment carried out from 1st April (in the 'grape' stage) until July, 1960, the greatest infestation of new buds again took place during the blossom period, i.e. between April 14th and 28th. In the immediate post-blossom period mites were still infesting new buds, but the rate was only half that during the blossom period. There was, however, another peak in the rate of infestation, from May 26th until June 1st, almost equal to that in the blossom period, but thereafter the rate fell away gradually until July. The experiment showed that some mites had been able to enter new buds successfully when liberated on or before the early 'grape' stage, and also when liberated as late as July, when control measures are commercially impracticable.

TABLE II.

EFFECTS OF SINGLE AND DOUBLE APPLICATIONS OF 1% LIME SULPHUR ON BLACK CURRANT
VARIETY BALDWIN.

Time of applications	Average % buds infested per block		% Reduction or increase		
	Pre-treatment	Post-treatment	Average	Max.	Min.
No spray	7.26	16.2	+145.0	+9.6	+237.1
April 7	9.15	8.7	4.3	37.5	+20.5
(Grape stage)					
April 7 + April 20	7.75	0.4	94.8	100.0	90.2
(Early blossom)					
April 7 + May 4	7.29	3.0	58.3	67.1	54.2
April 7 + May 18	7.15	5.0	29.5	62.8	+9.5
April 7 + May 31	5.78	3.4	40.3	86.0	7.2
April 7 + June 15	9.59	7.1	25.2	52.4	18.8

A spraying trial to find the optimum times for control measures was carried out on blocks of bushes of Baldwin at Long Ashton during 1960; the results are shown in Table II. Lime sulphur at a concentration of 1% was applied by hand lance to six of seven blocks of bushes at the 'grape' stage; each of five blocks was then given one other application at times coinciding with those of the previous trials. The level of infestation in the unsprayed bushes greatly increased, whilst the single 'grape' stage spray achieved a reduction of 4.3%, which approximated closely to that recorded in Table I. The 'grape' stage application plus one early-blossom spray gave an excellent average control and complete control in two bushes. The immediate post-blossom applications were not so effective: an application on May 31st was much more effective than those preceding and following it. From records of the major periods of infestation (4) it is clear why applications in the blossom period are the most effective and why an application at the end of May is also helpful.

During 1960 an experiment was made to find whether wettable sulphur had the same biological efficiency as lime sulphur. Sprays of wettable sulphur containing sulphur equivalent to that in 1% lime sulphur were applied six times to a block of Baldwin bushes, by hand lance, on the same dates as those on which the cumulative applications of lime sulphur were made. After six applications the average reduction in bud infestation was 94.2%: the maximum reduction was 95.0% and the minimum 89.0%. It will be seen that these results are very similar to those obtained after six applications of lime sulphur (Table I). Whilst a little leaf scorch was observed on the bushes which received more than four applications of lime sulphur, there appeared to be none on those on which wettable sulphur was used; further tests of wettable sulphur are being made.

It was found that satisfactory formulations could be made of lime sulphur, DDT and the fungicide zineb; whilst lime sulphur itself appeared to exert a useful check on the build up of leaf spot, the addition of zineb gave a much better control. The addition of DDT appeared to give a very satisfactory control of the Black Currant Leaf-curling Midge and capsid bugs and to keep aphids in check when the bushes had previously received a tar oil wash. Both lime sulphur and wettable sulphur prevented a build up of the Red Spider Mite

until late in the season. Further work is being done to determine how DDT and zineb can best be combined with sulphur sprays for the control of insect pests and leaf spot.

Summary.

- (1) Applications of the systemic chemicals, schradan, mevinphos, dimefox, demeton-methyl, dimethoate and fluoroacetamide to the soil before bud-burst and after fruit picking in 1959 failed to show any promise of controlling the Black Currant Gall Mite; foliar applications of these compounds made soon after bud-burst and after picking in the same year were also without significant effect.
- (2) In 1960 three applications of 1% lime sulphur by hand lance, at fortnightly intervals from the 'grape' stage, gave good control of the gall mite on the variety Baldwin without any apparent adverse effect on the bushes. In the same year, $\frac{1}{2}$ % lime sulphur gave equally good control on the variety Mendip Cross.
- (3) Lime sulphur sprays were most effective during the early blossom period and at one stage in the post-blossom period; these periods coincide with those at which mites have been found to penetrate new buds at the greatest rate.
- (4) A preliminary trial suggested that wettable sulphur may be as efficient as lime sulphur in controlling the gall mite and less liable to cause leaf scorch.
- (5) The incorporation of DDT and zineb in the sulphur sprays promised good control of various other pests and diseases of black currants.

Acknowledgments.

Thanks are due to Dr. C. G. L. Furnidge for assistance with chemical formulations, to Mr. C. P. Lloyd-Jones for the work on the uptake of radioactive phosphorus, and to Mr. A. A. Baker for help with spraying and recording.

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THE APPLICATION OF LIME SULPHUR FOR THE CONTROL OF THE BLACK CURRANT GALL MITE

By N. G. MORGAN and B. D. SMITH.

Introduction.

Studies of the conditions affecting the emergence of the Black Currant Gall Mite from the old infested buds ('big buds') and its subsequent behaviour (1) have suggested more effective methods for its control than those now in use.

Emergence begins at about the 'grape' stage and may continue until early July; during this extended period the mites are migrating and are able

to infest new axillary buds. Control depends upon preventing this infestation of new buds. Although the mite population on the bushes can be reduced by applying an acaricide to the old stems and infested 'big buds', it is essential that the material be also deposited in the leaf axils of the new growth. While new axillary buds are being formed, complete control of the mite is not possible unless the acaricide is present in the leaf axils throughout this period. This cannot be accomplished with a single application, however persistent the material used, because new unprotected buds would emerge after the spraying. Repeated applications are therefore essential.

In case it were necessary to make the final application when the fruits were nearing maturity, lime sulphur was chosen for the experiments; its residues are highly toxic to mites but have negligible mammalian toxicity and, at 1%, it has little phytocidal effect.

Experimental.

Preliminary Spraying Trials 1958-59.

A small scale field trial was carried out at Long Ashton in 1958. Twelve bushes of the variety Wellington XXX, with 20-64 per cent of the buds infested with gall mite, were sprayed six times with 1% lime sulphur and 0.025% dinonyl sodium sulphosuccinate wetting agent at fortnightly intervals from the 'grape' stage, using a hand lance. In the following autumn no sprayed bushes had more than 0.5% of the buds infested; on the unsprayed bushes the infestation varied between 47 and 85%.

Following these results a trial of large-volume full-coverage automatic spraying of lime sulphur was carried out at Luddington Experimental Horticulture Station in 1959. The main object of this trial was to assess the efficiency of repeated applications of lime sulphur for mite control: in order to ensure thorough spraying, drenching applications were made, using very much larger volumes of wash than would normally be applied in commercial practice.

A plot of mature bushes of the varieties Baldwin and Mendip Cross was divided into four blocks. Sixteen bushes in each block were selected for assessment of gall mite infestation in the spring before spraying and again in the following autumn. Three blocks were used for comparing the effect of six applications of 1% lime sulphur and 0.025% dinonyl sodium sulphosuccinate wetting agent, made at fortnightly intervals from the 'grape' stage both by hand lance and by automatic overhead boom, with a single application of 2% lime sulphur and wetting agent at the 'grape' stage only. The fourth block was left unsprayed.

The hand spraying was carried out at 250 p.s.i. flow pressure through a broom lance with three nozzles each with $\frac{1}{8}$ " discs. The overhead horizontal boom was a $\frac{3}{4}$ " copper pipe with two rows of $\frac{1}{4}$ " BSP nozzles spaced 9" apart, one row pointing forward and the other backward, each at 45° to the horizontal. The nozzles were fitted with $\frac{1}{8}$ " discs. The boom was mounted on a tubular steel supporting 'gate' 12" above the crop, and operated at 75 p.s.i. (Plate IIIb).

The infestation assessment carried out in the autumn showed that on the hand-sprayed block the reduction of galled buds was 94.6% and on the boom-sprayed block 87.6%. On the block sprayed once with 2% lime sulphur at the grape stage the reduction was 5.4% and on the unsprayed block 7.3%.

PLATE III.



(a) Vertical mast.



(b) Overhead boom.



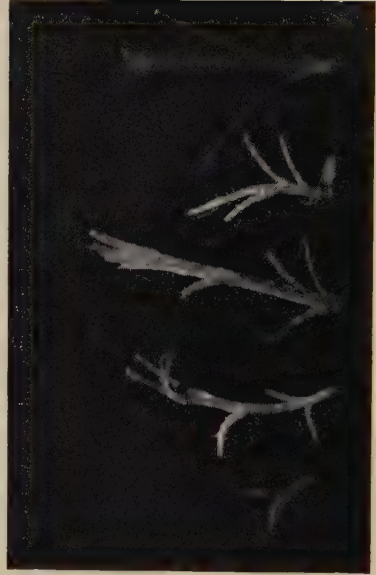
(c) Compressed air.

Machines used in black currant spraying trial, Long Ashton, 1960.

PLATE IV.



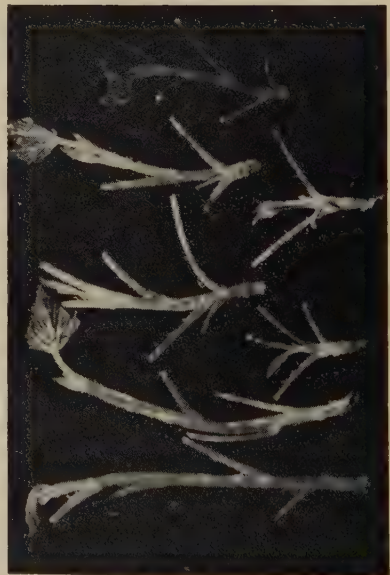
(b) Large-volume vertical mast.



(d) Small-volume compressed air.



(a) Large-volume hand lance.



(c) Large-volume overhead boom.

Ultra-violet photographs of fluorescent tracer deposits on black currant shoots; right-hand shoot unsprayed.

Spraying Trial : 1960.

In 1960 an experiment was carried out at Long Ashton to compare the effect on gall mite infestation of lime sulphur applied at regular intervals by the following methods :—

Large-volume spraying of 1% lime sulphur at 200 gal. per acre.

- a. Lance spraying.
- b. Automatic spraying with vertical masts.
- c. Automatic spraying with horizontal boom.
- d. Compressed air atomization.

Small-volume spraying at 20 gal. per acre.

- e. Compressed air atomization of 1% lime sulphur.
- f. Compressed air atomization of neat lime sulphur.

These methods were compared with a single application of 2% lime sulphur by hand lance at the 'grape' stage only.

The lance spraying was carried out with a single nozzle at 75 p.s.i. and was timed for each bush so that the application rate of 200 gal. per acre was maintained as accurately as possible. The vertical mast automatic spraying was carried out with the machine described by Kearns and Morgan (2). To this machine was attached the overhead boom previously used in the Luddington experiment; the nozzles were fitted with 5/64" discs. The tractor-mounted machine used for atomization consisted of a tank, liquid feed pump and a rotary compressor delivering air at approximately 75 p.s.i. to six twin-fluid swirl-mixing nozzles designed and described by Cobbald (3). The nozzles were mounted on a horizontal tubular steel boom and were arranged to spray a single row of bushes from above and from each side (Plate IIIc). The required flow-rate to the nozzles for 200 or 20 gal. per acre was selected by means of valves and a flow meter.

The dilute lime sulphur was used in combination with dinonyl sodium sulphosuccinate wetter at a concentration of 0.025%, in order to obtain the greatest possible cover of the stems and leaf axils.

A block of 24 mature bushes of the variety Baldwin was used for each method and sprayed at six fortnightly intervals from the 'grape' stage. One block of bushes was sprayed with 2% lime sulphur and wetting agent at the 'grape' stage only. A block of 38 bushes was left unsprayed. In order to obtain a visual assessment of spray distribution a fluorescent dye was applied with the same machines under the same conditions. Interaction with the dye mixture prevented direct tracing of the lime sulphur so it was necessary to spray adjacent bushes with the dye mixture and wetting agent only.

Results.

Gall Mite Control.

The buds on every bush were counted before spraying and in the following autumn. The first count was of buds formed during the previous season, the second of buds formed in the current season. Table I shows the results expressed as the average percentage infestation before and after spraying, the average percentage reduction in infestation resulting from the treatments, and also the maximum and minimum effect of each treatment.

TABLE I.

PERCENTAGE MITE INFESTATIONS OF BUDS OF BALDWIN BLACK CURRANTS: 1960.

(Means for 24 bushes)

Lime Sulphur applications			Percentage buds infested		Percentage reduction (—) or increase (+) in infested buds		
Strength	Method	No.	Pre-treat- ment	Post-treat- ment	Mean	Max.	Min.
1%	LV Hand Lance	6	13.45 (± 3.9)	1.00 (± 1.0)	—92.5	—98.0	— 65.1
1%	LV Vertical mast	6	13.23 (± 3.6)	1.67 (± 0.8)	—87.1	—96.6	— 85.9
100%	SV Compressed air	6	19.34 (± 8.5)	3.63 (± 0.7)	—81.3	—83.9	— 75.9
1%	LV Compressed air	6	10.48 (± 4.5)	2.99 (± 2.8)	—71.1	—86.6	— 7.5
1%	LV Overhead boom	6	13.26 (± 3.7)	4.56 (± 2.3)	—65.9	—83.1	— 41.5
1%	SV Compressed air	6	9.76 (± 6.2)	12.40 (± 5.1)	+26.9	—71.3	+274.4
2%	LV Hand lance	1	9.50 (± 3.2)	8.90 (± 2.7)	—6.3	—30.0	+23.1
—	Unsprayed	—	*13.90 (± 3.4)	13.00 (± 2.7)	— 6.4	—65.6	+129.4

LV=Large-volume (200 gal./acre). SV=Small-volume (20 gal./acre). *33 bushes.

The outstanding results are the large reduction in infested buds caused by the large-volume hand-lance applications of 1% lime sulphur made at fortnightly intervals, and the failure of the single large-volume hand-lance application of 2% lime sulphur at the 'grape' stage. The large-volume automatic spray applications of 1% lime sulphur reduced infestation, but the small-volume applications failed. The small-volume applications of neat lime-sulphur gave a high degree of control but caused severe phytocidal damage and crop reduction.

Fluorescent Tracer Deposits.

Photographs taken in ultra-violet light of representative samples of shoots from bushes sprayed with Primulin A, a water-soluble fluorescent dye, with added succinate wetting agent, are shown in Plate IV. Intense fluorescence was found on a greater number of axillary buds on the bushes sprayed by the large-volume hand-lance, vertical mast and compressed air methods than on those sprayed by the other methods. The small-volume compressed air method gave a uniformly distributed deposit which was, however, much less dense than that given by the other methods.

Discussion.

These field trials show that the Black Currant Gall Mite was controlled by six applications of 1% lime sulphur and 0.025% succinate wetter without apparent damage to the bushes. A single application of 2% lime sulphur and wetter at the grape stage failed to control the mite. Further trials are required to determine the efficacy of a smaller number of applications (4), of improved small-volume spraying methods and of a reduced concentration of lime sulphur. Further work is also required to establish the relative importance to mite control of lime sulphur spray deposits on the old buds and stems and those in the leaf axils of the new growth.

Summary.

Preliminary trials in 1958 and 1959 showed that six drenching applications of 1% lime sulphur with added wetting agent, made at fortnightly intervals from the 'grape' stage, gave excellent control of the Black Currant Gall Mite.

In 1960 different methods of large-volume spraying of 1% lime sulphur and wetter at 200 gallons per acre were compared with small-volume spraying at 20 gallons per acre. Large-volume hand-lance spraying gave excellent control; vertical mast automatic spraying was only slightly less effective. Large-volume applications by compressed air nozzles were slightly more effective than the same volume applied by overhead horizontal boom. Small volumes of 1% lime sulphur applied by compressed air nozzles were ineffective. Small-volume applications of neat lime sulphur by compressed air nozzles gave good control of the mite but badly damaged the bushes.

Acknowledgments.

The authors are indebted to the Director and Staff of the Luddington Experimental Horticulture Station for providing facilities for a field trial and to Messrs. T. E. Cobbald and J. H. Russell for assistance with the design and operation of spray machinery; to Mr. A. A. Baker for biological assays and to Mr. H. R. Mapother and Miss B. K. I. Bird for photographs.

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THE USE OF RADIOACTIVE PHOSPHORUS TO FOLLOW THE MOVEMENT OF THE BLACK CURRANT GALL MITE.

By C. P. LLOYD-JONES and B. D. SMITH.

In studying the movement of the Black Currant Gall Mite (1,2) attempts were made in 1959 to label mites with the radioactive isotope of phosphorus, ^{32}P . This isotope was chosen because of its convenient half-life (14 days) and ease of measurement; it was used as a 10 p.p.m. solution of phosphorus in the form of sodium dihydrogen phosphate at a specific activity of 1 mC ^{32}P /10 mg. P.

Attempts to label mites by placing shoots cut from black currant bushes before bud-burst in ^{32}P solution were not successful. Little liquid was taken up by the shoots until the buds opened, and external scanning with a thin end-window type G.M. tube monitor then showed radioactivity in the healthy expanding buds but not in those galled, which usually failed to develop leaves. An alternative technique of injecting galled buds with ^{32}P solution by hypodermic syringe was therefore tried. After 3 days mites dissected out were shown by scanning with a monitor to be radioactive; it was possible to get 100 counts/min. from approximately 2000 mites. Washing the mites in a radio-

inactive phosphate solution of 10 p.p.m. P indicated that the ^{32}P was not present as a contaminant that could be easily lost, although it was not determined how it had entered the tissues of the mites.

To find the direction of movement of mites after emergence from infested buds, galled buds were injected with ^{32}P solution, cut off after 12 hours, and attached to healthy plants with small wire hooks. The mites emerged rapidly from the infested buds but it proved impossible to detect their presence by a monitor after dispersal. They could, however, be detected by taking tissues from the plant, digesting them in nitric acid, making the digest to a standard volume and counting the radioactivity in a liquid M6 type G.M. tube. By this procedure the mites were found to move preferentially upwards from the point of application and to tend to congregate in the leaf axils of the host plant.

TABLE I.

PERCENTAGE DISTRIBUTION OF ^{32}P ON SHOOTS AFTER ATTACHMENT OF INJECTED GALLED BUDS.

Interval from attachment to sampling	Shoot above bud	Shoot and leaf axil tissue below bud	Leaf axil tissue above bud
1 day	64	36	—
3 days	76	22	2
6 days	54	—	46
8 days	68	—	32

The technique has helped to show the direction of movement of mites (1,2), but a close correlation was not obtained between the radioactivity recorded and the numbers of mites determined by dissection of the tissues, probably because of variations in the radioactivity associated with individual mites. The radioactivity appeared not to affect the behaviour of the mites during migration or their penetration of axillary buds and subsequent reproduction.

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"NON-FERMENTABLE" SUBSTANCES IN CIDERS AND PERRIES.

By G. C. WHITING.

Introduction.

Both cider-apple and perry-pear juices are now usually fermented to dryness, that is until all the fermentable sugars have been used by the yeasts present. Of the many substances that occur in ciders and perries some are present in the original juice while others are products of microbial or chemical action. In some years, many perries still show high specific gravity when fermentation has apparently ceased. Although it has been shown that the presence of sorbitol can account for some of this high residual gravity, the exceptionally high values found in 1959 required further investigation. The substances present after fermentation of ciders and perries were therefore studied to determine their identity, the amounts present, and their contribution to the specific gravity. Non-volatile organic acids have been previously described (1, 2), but for completeness the contributions of malate and citrate towards specific gravity have also been measured.

Experimental Methods.

The specific gravity of each juice, cider and perry was determined by using an accurate hydrometer, while the gravity of other solutions was obtained by weighing a known volume. All determinations were made at 20°C. To conform with convention, the absolute specific gravities are here multiplied by 1,000 and the units in excess of 1,000 are referred to as "degrees of S.G." Titratable and total acidities were determined as previously described (1). "Tannin" was determined by direct titration with potassium permanganate, using indigo-carmin as indicator (see page 143).

For many of the determinations it was necessary to remove acids and basic materials by passing the solution first through a column of Amberlite resin IR-120 (hydrogen form) and then through a column of Amberlite resin IRA-400 (carbonate form).

Qualitative Determination of Carbohydrates.

The cider or perry, after ion-exchange resin column treatment as described above, was treated with lead acetate solution, which removed a high proportion of "tannin". The clear solution was subjected to paper chromatographic examination using Solvent A: *n*-butanol-ethanol-water (10-1-2). The spray reagents used were aniline hydrogen phthalate, *p*-anisidine hydrochloride (for the detection of sugars), borax-boric acid with bromocresol purple (for the detection of hexitols) (3), and Trevelyan's silver nitrate dipping reagent followed by ethanolic sodium hydroxide spray (for the detection of sugars, polyols and glycerol) (4). Authentic specimens were run on the same chromatogram for comparison of R_F values and spray reactions.

Quantitative Determination of Carbohydrates.

A paper chromatographic method similar to that previously described for acids (1) was used to determine quantitatively glycerol, hexitols and sugars.

Glycerol was determined after spotting 4 μ l of cider or perry, after ion-exchange resin treatment, together with standard spots of 10 μ g, 20 μ g, 30 μ g, 40 μ g and 50 μ g of glycerol on the starting line, and running the chromatogram overnight in Solvent A. The glycerol spots were revealed by the use of Trevelyan's reagent (4).

A similar method was used for hexitols with standards of 10 μ g-50 μ g of sorbitol. The chromatogram was run for two days and sprayed with borate-bromo-cresol purple (3). The solvent used does not separate sorbitol, mannitol and dulcitol but in the absence of a malo-lactic change the hexitol has been assumed to be sorbitol. Fructose was determined using Trevelyan's reagent while, for glucose and xylose, *p*-anisidine was used.

An approximate value for the sorbitol content of perries was obtained, without preliminary ion-exchange treatment, after running the chromatogram with standards of 50 μ g-200 μ g for two days. The paper was then sprayed with 1% *p*-anisidine hydrochloride in water-saturated *n*-butanol, heated ten minutes at 100°C and then dipped in a mixture of one volume of an aqueous saturated solution of sodium metaperiodate and four volumes of acetone. Sorbitol was revealed as a white spot on a purple background (5).

Volumetric Determination of Sorbitol.

Sorbitol was accurately determined in perries by titration of the formic acid produced on oxidation with periodate. An aliquot of perry was passed through ion-exchange resin columns as described above and finally diluted to 100 times its original volume with distilled water. 2 ml. of 0.1 M sodium metaperiodate solution were added to 5 ml. of the diluted perry in a flask which was then stoppered and allowed to stand 3 hours at room temperature. 5 ml. of 10% aqueous ethylene glycol were added to decompose excess periodate and the mixture was allowed to stand 10 minutes. The formic acid produced was titrated with 0.01 N sodium hydroxide solution to the methyl red end-point using a stream of CO₂-free air; a blank determination was done simultaneously. Corrections were applied for the glycerol and sugars present; glycerol gave the theoretical yield of formic acid; glucose, xylose and fructose gave 61%, 56% and 42% respectively of the theoretical yield. A small correction was applied for the acid produced by the action of periodate on the tannin present in perry. Preliminary treatment with nylon powder, which quantitatively removed leucoanthocyanins, made this correction unnecessary.

Results and Discussion.

Non-fermentable Sugars in Dry Ciders.

The main carbohydrate constituents in most dry ciders were hexitol and glycerol. In sulphited fermentations sorbitol present in the original juice remained unchanged. In juices fermented by the natural flora, mannitol, which would be determined together with sorbitol by the methods used, may be formed by the heterofermentative lactic acid bacteria during fermentation. The range of hexitol contents found was 0.35%-0.75%. In some ciders that had been fermented by the natural juice flora, no glycerol was detected, but amounts up to 0.5% were found in sulphited ciders.

The main "non-fermentable" sugar was D-xylose (6)—about 0.05% in most ciders. But in some ciders fermented with the natural flora xylose was

absent, although detectable in the original juices: in these ciders it had evidently been metabolised, probably by lactic acid bacteria. Other sugars detected in trace amounts and tentatively identified by the paper chromatographic methods used were galactose, arabinose, ribose, rhamnose, sorbose and inositol (about 0.04%); in addition several oligosaccharides were detected: the main ones had R_G values (R_F values relative to that of glucose) in Solvent A of 0.17 and 0.40. Arabinose and galactose may have been formed by enzymic hydrolysis of the araban and galactan associated with the pectic substances of the juice. These are demethylated by a methylesterase present in the fruit and then degraded by yeast polygalacturonase (7) to mono-, di- and trigalacturonic acids; these acids have been isolated from ciders (2). It is possible that some trace sugars may have appeared from hydrolysis of flavonoid glycosides during the fermentation; galactose, glucose, rhamnose, arabinose and xylose have been found in the glycosides of quercetin or cyanidin in the apple (8). Ribose may have been liberated from autolysed yeast cells.

Many of the trace sugars and the oligosaccharides disappear during storage of the ciders, most probably as a result of bacterial action. The total amount of trace sugars at dryness is usually about 0.1% (excluding sugar acids).

Non-fermentable Sugars in Perries.

The trace sugars are more difficult to detect by paper chromatographic means in dry perries than in ciders because of the higher content of sorbitol in perries; values from 1.0% to more than 5.0% have been found. Glycerol occurs in amounts similar to those in ciders while xylose has sometimes been found in larger amounts, up to 0.2%. Both glycerol and xylose have been observed to be metabolised, probably by lactic acid bacteria, during storage of perry. Trace sugars tentatively identified were galactose, arabinose, ribose and inositol (about 0.04%), together with an oligosaccharide (R_G 0.05 in Solvent A).

The Contribution of Non-fermentable Substances to the Residual Gravities in Dry Ciders and Perries.

The difference between the specific gravities of juice and dry cider or perry may be used to calculate the amount of alcohol formed (decrease in degrees of S.G. $\div 7.5$ = per cent alcohol by volume). The gravity of the corresponding alcohol-water mixture (obtained from tables) subtracted from the gravity of the dry cider or perry then gives the degrees of gravity to be accounted for by other constituents. Table I shows the degrees of gravity found for 1% (w/v) solutions of a number of these constituents; those asterisked were obtained from tables, the rest by laboratory determination. The citric and malic acid solutions were brought to the specified pH values by adding KOH; the tannin was a leucoanthocyanin from perry.

The usual range of degrees of gravity to be accounted for in dry cider is 6°–12°; in practice, if a cider fermentation ceases at a gravity greater than 1005°, fermentable sugar is still present. The residual gravity of a dry cider is accounted for by sorbitol, glycerol, tannin and acid, free and combined.

Perry fermentations may cease at a gravity much higher than 1005°. In

TABLE II.
RESIDUAL GRAVITY IN PERRIES.

Variety	Original Gravity of Juice	Final Gravity of Perry	Degrees Gravity to be accounted for	% Sorbitol	% Tannin	% Fructose	% Glucose	% Xylose	% Glycerol	% Total Acidity	Degrees Gravity accounted for
Rumblers	1070.6	1014.2	20.2	2.75 <i>9.63</i>	0.32 <i>1.70</i>	0.08 <i>0.35</i>	—	0.1 <i>0.42</i>	0.63 <i>1.51</i>	1.28 <i>6.91</i>	20.5
Taynton Squash ..	1094.4	1015.4	23.4	3.25 <i>11.38</i>	0.24 <i>1.27</i>	0.2 <i>0.84</i>	Trace	0.1 <i>0.42</i>	0.88 <i>2.11</i>	1.64 <i>8.36</i>	24.4
Brown Huffcap ..	1104.2	1025.4	33.4	5.04 <i>17.64</i>	0.84 <i>4.45</i>	0.03 <i>0.12</i>	Trace	Trace	0.83 <i>1.99</i>	1.22 <i>7.56</i>	31.8
Thorn	1088.5	1009.4	17.4	2.97 <i>10.40</i>	0.34 <i>1.80</i>	0.1 <i>0.42</i>	Trace	Trace	0.84 <i>2.02</i>	1.09 <i>5.89</i>	20.5
Oldfield	1088.1	1023.7	30.7	4.40 <i>15.40</i>	0.34 <i>1.80</i>	0.4 <i>1.68</i>	0.2 <i>0.8</i>	0.05 <i>0.21</i>	0.76 <i>1.82</i>	1.84 <i>9.57</i>	31.3
Rock	1091.1	1017.3	25.3	3.86 <i>13.51</i>	0.70 <i>3.71</i>	0.1 <i>0.42</i>	Trace	0.1 <i>0.42</i>	0.66 <i>1.58</i>	0.96 <i>5.95</i>	25.6

Values in italics represent degrees of gravity calculated from Table I.

TABLE I.
DEGREES OF GRAVITY OF 1% SOLUTIONS OF
JUICE CONSTITUENTS.

Fructose	4.2*
Sorbitol	3.5
Glycerol	2.4*
Citric acid : pH 3	4.6
Citric acid : pH 4	5.6
Malic acid : pH 3	4.6
Malic acid : pH 4	6.2
Tannin	5.3

the seasons 1955-58, 75 perries were fermented and their final gravities determined; the final gravity of one perry was 1021°, ten were in the range 1010°-1020° while the remainder were below 1010°. Sorbitol contents of up to 3% were found in the high gravity perries. In the 1959 season the final gravities of 65 perries were determined; seven were greater than 1020° and fifteen were in the range 1010°-1020°. Six of these high gravity perries were examined in detail. Each of the main perry constituents was determined and its contribution to the gravity calculated from Table I. The results are shown in Table II. The sum of the individual contributions to the gravity (final column Table II) agreed well with the "degrees of gravity to be accounted for" described above. Sorbitol and acid made the main contributions: tannin and glycerol were rather less important. The unusually high sorbitol and acid contents were presumably due to the hot dry growing season; dehydration of the fruit may also have been contributory.

Summary.

- (1) The trace sugars in dry ciders and perries have been tentatively identified.
- (2) Methods are described for the determination of glycerol and sorbitol in ciders and perries.
- (3) The contribution of sorbitol, leucoanthocyanin, citric and malic acids to the gravity of dry cider or perry have been determined and the high final gravities of certain dry perries have been explained.

Acknowledgments.

The author wishes to thank Mrs. G. M. Hunt and Mr. J. H. Llewellyn for valuable assistance.

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THE OPTICAL ROTATION OF PERRY PEAR LEUCOANTHOCYANIN.

By L. F. BURROUGHS and G. C. WHITING.

Introduction and Experimental.

During the course of the work described in the previous paper (1), it was thought that determination of the optical rotation of a perry might help to detect the presence of unfermented sugar, or more specifically of fructose, which is usually the last sugar to be fermented.

Of 23 perries examined, only one showed a negative rotation indicating the presence of more than 0.25% fructose, while ten perries had a positive rotation ranging from 0.03° to 1.08° (5 cm. tube).

The positive rotation was shown to be due to a neutral substance, for it was still given after the removal of acids and bases by ion-exchange. After passage through cation (Amberlite IR 120-H⁺ form) and anion (Amberlite IRA 400-acetate form) exchange columns, the perry showed in fact a very small increase in rotation because of the removal of laevo-rotatory chlorogenic and malic acids. The rotation was not due to glucose, as paper chromatographic examination showed that all sugars except sorbitol (weakly laevo-rotatory) were virtually absent. It appeared, however, to be correlated with the "tannin" content of the perry as determined by potassium permanganate titration (see Appendix). Table I shows the optical rotations and "tannin" contents of a number of perries (both determined after passage through cation- and anion-exchange resin columns and after correction of the rotation for residual sugars). The figures are also included in Fig. 1.

TABLE I.
OPTICAL ROTATION AND TANNIN CONTENT OF PERRIES.

Perry			Rotation		"Tannin"*	
Ref. No.	Variety		(5 cm.-tube) in degrees	%	m. eq. KMnO ₄ per 100 ml. of perry	
I 11	Rumblers		+0.32	0.32	8.0	
VII 16	Taynton Squash		+0.19	0.24	6.0	
VII 30	Rock		+1.13	0.88	22.0	
VII 35	Brown Huffcap		+1.05	0.84	21.0	
VII 40	Thorn		+0.32	0.34	8.5	
VII 45	Oldfield		+0.33	0.34	8.5	
VII 67	Rock		+0.80	0.70	17.5	

*The two methods of expressing tannin content are explained in the Appendix.

Williams (2) has shown that the permanganate-reducing substances ("tannin") in perry pears are mainly leucoanthocyanins with a little chlorogenic acid and a trace of catechin. The "tannin" contents shown in Table I

are therefore a measure of their leucoanthocyanin content, as chlorogenic acid would have been removed by the resin column treatments. Since polyamide resins are selective adsorbents for the leucoanthocyanins present in beer (3), one perry (VII 67) was shaken for 20 minutes with one-tenth of its weight of nylon 66 powder. This treatment decreased the rotation by 0.58° and the tannin titre by 13.25 m. eq. per 100 ml. Two similar successive treatments of perry VII 30 decreased the rotation by 1.06° and the tannin titre by 19.75 m. eq. per 100 ml. In view of this evidence for the optical activity of perry leucoanthocyanins, two methods were tried for the separation of these compounds.

According to Vuataz *et al.* (4), leucoanthocyanins are completely precipitated by lead acetate at pH 5.5 and can be regenerated by treating the lead precipitate with cation exchange resin (H^+ form). Sodium hydrosulphite (100 mg.) was added to 100 ml. of perry (VII 30) to minimize oxidation during manipulation. The perry was adjusted to pH 5.5 with NaOH, and 7.5 ml. of saturated lead acetate solution was added dropwise with thorough stirring. The heavy cream-coloured precipitate was centrifuged and washed twice with slightly acidified (acetic) water. The deposit was suspended in 25 ml. of 10% ethanol and treated with 7.5 g. of Zeokarb 225 (H^+ form) to adsorb the Pb^{++} ions and so liberate the polyphenols. The resin was filtered off and washed, yielding in all 45 ml. of pale reddish-orange solution (A) with a tannin content equivalent to 10.1 m. eq. of $KMnO_4$. This represents 44% of the tannin present in the original 100 ml. of perry (23 m. eq.). The solution also contained 8.1 m. eq. of acid, shown by paper chromatography to be almost entirely malic acid, representing 40% of the total malate content of the original perry (20.4 m. eq.). Because of the rather low recovery of polyphenols in the above procedure and the concomitant precipitation of malate, another method of precipitation was tested.

During attempts to isolate protein from apple juice by precipitation as the zinc complex (5) it was found that at a slightly alkaline pH much of the polyphenolic material present was also precipitated. These conditions were therefore used with perry VII 30. As before, 100 mg. of sodium hydrosulphite was added to 100 ml. of centrifuged perry and the alcohol content was increased to 25% by volume. Zinc sulphate (4 milli-moles) was then added and the pH raised to 7 by adding 2.25 ml. of *N* NaOH. The yellowish precipitate obtained was centrifuged and washed with water (precipitate B).

The centrifugate at pH 7 was brought to pH 7.5 and gave a further bulky gelatinous buff precipitate. This was centrifuged and washed with 20% ethanol (precipitate C).

Precipitates B and C were suspended in 10 ml. and 25 ml. respectively of 10% ethanol and treated with small portions of Zeokarb 225 (H^+ form). The resin was filtered-off and washed, yielding 10 ml. and 45 ml. respectively of reddish-brown solution. Filtrate B possessed only 2% (0.46 m. eq.) of the total permanganate reducing power of the original perry (23 m. eq.) but filtrate C retained 63% (14.6 m. eq.). The acidity of filtrate C was only 9% of the total acidity of the perry and was thus much lower than that obtained from the lead precipitation method.

Solution D was obtained by a double zinc precipitation followed by passage of the solution through cation and anion-exchange columns.

The optical rotations (5 cm. tube) and the $KMnO_4$ -reducing values of the various leucoanthocyanin solutions were determined and are shown in Table II.

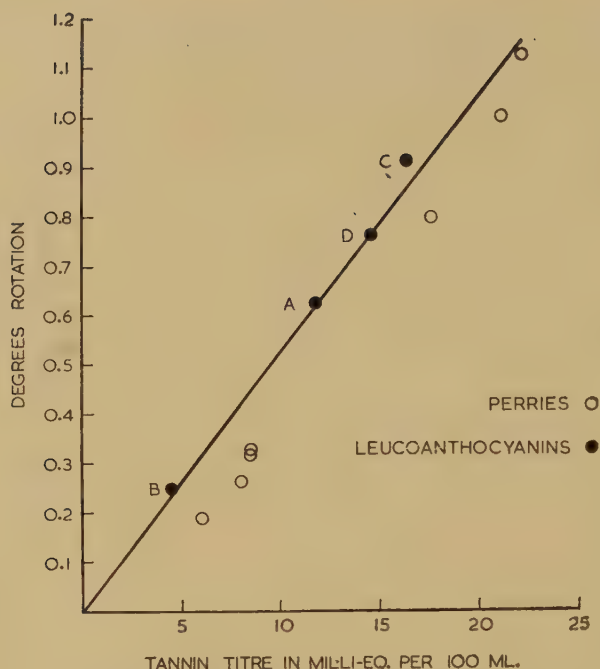


Fig. 1. Optical rotation and tannin titre of perries (Table I) and perry leucoanthocyanins (Table II)

TABLE II.
OPTICAL ROTATION OF LEUCOANTHOCYANIN SOLUTIONS.

Solution	Rotation	KMnO ₄ value (m. eq. per 100 ml.)
A	+0.625°	11.5
B	+0.25°	4.5
C	+0.914°	16.25
D	+0.764°	14.5

The traces of malic acid in solutions A, B and C and of chlorogenic acid in Solution A would have a negligible effect on the optical rotation. The values of Table II, when plotted in Figure 1, clearly show that perry leucoanthocyanin is strongly dextro-rotatory and that the rotation is directly proportional to the leucoanthocyanin content.

When 10 ml. of solution D were evaporated to dryness in a vacuum desiccator a dry weight of 0.123 g. was obtained. If it is assumed that the basic formula for perry leucoanthocyanin, as suggested by its conversion to cyanidin with HCl (6), is 5,7,3',4'-tetrahydroxyflavan-3,4 diol, and if it is also assumed that this basic unit consumes two atoms of oxygen on oxidation with KMnO₄,

it may be calculated that the concentration in Solution D is 1.11% w/v. This agrees reasonably well with the dry weight determination. The leucoanthocyanin content of Solution D is therefore approximately twice the "tannin" content (0.58%) expressed by the convention hitherto used for cider.

From the optical rotation and KMnO_4 -reducing value of Solution D, the specific rotation of perry leucoanthocyanin is calculated as

$$[\alpha]_{\text{D}}^{20} = +138^\circ.$$

Appendix.

Lowenthal Titration of "Tannins".

The method used at Long Ashton is similar to the method of the Association of Official Agricultural Chemists (7) but is carried out on a smaller and more convenient scale. Only a direct determination of total permanganate-reducing power is made, in view of the uncertain specificity of the methods for precipitating tannins, e.g. by treating with gelatine, charcoal etc. (8).

1 ml. of sample (juice, cider or perry) is pipetted into 200 ml. of water containing 5 ml. of indigo carmine indicator and titrated slowly with $N/40 \text{ KMnO}_4$ (freshly diluted from $N/10$ stock solution) to a green colour and then dropwise until just yellow. If the titration is more than about 5 ml. it is repeated using only 0.5 ml. of sample.

The indigo carmine indicator (containing 50 ml. H_2SO_4 conc. per litre) is diluted from stock solution (7) so that 5 ml. is exactly equivalent to 1 ml. of $N/40 \text{ KMnO}_4$. This indicator blank is subtracted from the titration to give the KMnO_4 reduced by the tannins. This value (ml. $N/40 \text{ KMnO}_4$ per ml. cider) divided by 10 gives the tannin content (gm./100 ml.) in the conventional manner.

This calculation assumes that 1 ml. $N \text{ KMnO}_4$ is equivalent to 40 mg. of tannin, compared with 41.6 mg. in the A.O.A.C. method. The difference is, however, within experimental error in view of the uncertainty of the end-point. Both methods, being based on the original method of Lowenthal, are referred to an arbitrary standard of tannic acid. This is unfortunate in the light of present knowledge of the more complex nature of fruit tannins (2, 8) but results are comparable and conform with long established tradition in cider- and wine-making.

From a more fundamental point of view, it is preferable to calculate the results simply in terms of permanganate-reducing value expressed as milliequivalents of KMnO_4 (ml. of $N \text{ KMnO}_4$) for a given volume of sample.

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THE SULPHUR DIOXIDE COMBINING POWER OF CIDER.

By L. F. BURROUGHS and G. C. WHITING.

When SO_2 is added as a preservative to cider, part of it combines with carbonyl compounds* present and so becomes ineffective against bacteria. In some ciders the amount of SO_2 bound in this way may be so great as to make it impossible to retain sufficient free SO_2 to be an effective safeguard against infection within the legally prescribed limit of 200 p.p.m. total SO_2 ; the nature and amount of the SO_2 -binding compounds in cider has therefore a great practical significance. The investigation of this problem is still in progress and much remains to be done, but the results already obtained are thought to be sufficiently important to warrant the publication of this progress report.

Sulphur dioxide has been used for many years as a preservative in fruit and vegetable products, and many investigations have been made of free and bound SO_2 in such materials (1,2). The picture is, however, very complex and remains confused. The recent work of Kielhöfer and Wurdig (3,4) on wines is a systematic attempt to account for the bound SO_2 in terms of the carbonyl compounds known to be present (mainly acetaldehyde and glucose). There is, however, always a residuum of SO_2 -binding power attributable to unidentified substances, which in some wines may be as high as 100 p.p.m. A very similar situation exists in cider.

SO_2 -Binding Power.

The SO_2 -binding power of a cider, at a given temperature and pH, is determined by the concentration of each of the SO_2 -binding substances present and by the dissociation constants of their respective bisulphite compounds. It should be noted that because the combination of a carbonyl compound with SO_2 is an equilibrium reaction, the composition of the equilibrated system depends on the concentration of free SO_2 . Hence the SO_2 -binding power of a given solution can be stated only with reference to a selected free SO_2 content. For the sake of uniformity, 50 p.p.m. free SO_2 has been used throughout this work; this value has practical significance since it appears to give adequate protection against normal chance infection of the cider with micro-organisms.

In ciders, as in wines, acetaldehyde is quantitatively the most important SO_2 -binding constituent. Its affinity for SO_2 is so great that, if acetaldehyde is present in only small excess, virtually the whole of the SO_2 becomes bound; conversely, if the SO_2 is in excess, the whole of the acetaldehyde is bound. In a dry cider from a non-sulphited fermentation the acetaldehyde content is generally in the region of 10-20 p.p.m., equivalent to 15-30 p.p.m. SO_2 . In a cider from a sulphited fermentation, the whole of the SO_2 becomes bound and there may be a few p.p.m. of free acetaldehyde. In either case, free acetaldehyde does not account for more than 30 p.p.m. of SO_2 -binding power. Most ciders, however, can bind 50-80 p.p.m. SO_2 (i.e. in addition to SO_2 bound during a sulphited fermentation) and some have been found to bind much more. Only

*The term carbonyl compound is used in this paper to mean a substance which reacts with SO_2 to form a hydroxysulphonic acid, i.e. a "bisulphite compound".

a part of this can be attributed to glucose which, although present at concentrations up to 2% in sweet ciders, has such a low affinity for SO_2 that not more than 10 p.p.m. can be accounted for in this way. The difference must be attributed to other SO_2 -binding substances, some of which are considered below.

Recent work at Long Ashton on carbonyl compounds in ciders, particularly those fermented in the presence of SO_2 , has shown the presence of mono, di- and trigalacturonic acids (5), pyruvic acid and α -ketoglutaric acid (6) and xylosone (7). The dissociation constants of the bisulphite compounds of these substances and also of acetaldehyde have been determined, at a temperature of 20°C and in the pH range 3–4. These are calculated in the usual way from the Law of Mass Action, with the free- SO_2 term including all forms of free SO_2 and not merely the bisulphite ion. The value for xylose-bisulphite is taken from the data of Sundman at 19°C and pH 4.65, quoted in Ref. 1, p. 74; that for glucose-bisulphite is taken from Vas (8). Using these constants, the SO_2 -binding power attributable to each of these substances, at the concentration at which it occurs in cider, can be calculated (see Table I).

TABLE I.

THE SO_2 -BINDING POWER OF CARBONYL COMPOUNDS OCCURRING IN CIDER.

Carbonyl compound	Normal range of concentration in cider %	Dissociation constant K	SO_2 -binding power (at 50 p.p.m. free SO_2)	
			Bound carbonyl compound as % of total	Bound SO_2 (p.p.m.)
Acetaldehyde	0.002—0.010	1.5×10^{-6}	100	29–145
Pyruvic acid	0.000—0.020	4.0×10^{-4}	66	0–96
α -Ketoglutaric acid ..	0.000—0.003	8.8×10^{-4}	47	0–6
L-Xylosone	0.000—0.020	2.1×10^{-3}	27	0–23
Monogalacturonic acid	0.040—0.200	3.0×10^{-2}	2.5	1–16
Trigalacturonic acid ..	0.010—0.200	3.7×10^{-2}	2.1	0–5
Xylose	0.000—0.050	6.9×10^{-2}	1.1	0–2
Glucose	0.0 —1.8	6.4×10^{-1}	0.12	0–8

Acetaldehyde. As indicated previously, the concentration of acetaldehyde in ciders can vary widely, depending on how much SO_2 was added to the juice before fermentation; a juice with 150 p.p.m. SO_2 will produce a cider with about 100 p.p.m. acetaldehyde. The value of the dissociation constant in Table I is taken from data obtained in an extensive study of the acetaldehyde-bisulphite system, which will be reported elsewhere. This value (1.5×10^{-6}) is slightly lower than the usually quoted value (2.4×10^{-6}) of Kerp (9) and also lower than the recently reported value (2.8×10^{-6}) of Diemair, Koch and Hess (10).

Pyruvic Acid. Of the remaining carbonyl compounds, pyruvic acid is much the most important SO_2 -binding species, and is probably responsible for much of the hitherto unidentified bound SO_2 in certain ciders. Its presence is particularly important in that the free pyruvic acid in equilibrium with the system is capable of binding more SO_2 if a further increment of SO_2 is added. For example, a cider with a total pyruvic acid content of 100 p.p.m. and free

SO₂ content of 20 p.p.m. would contain 44 p.p.m. of bound pyruvic (equivalent to 32 p.p.m. bound SO₂) ; to achieve 50 p.p.m. free SO₂ one would need to add, not 30 p.p.m. but 44 p.p.m. SO₂, the extra 14 p.p.m. being required to combine with the free pyruvic acid. All ciders with a high pyruvic acid content, so far examined, also have a high SO₂-binding power, but not all of this can be accounted for as pyruvate-bisulphite, i.e. other SO₂-binding substances are also present.

α-Ketoglutaric Acid. The dissociation constant of *α*-ketoglutaric acid is of the same order as that of pyruvic acid, but the amounts present in ciders are usually much less. However, one cider made from concentrated juice was found to contain 70 p.p.m., capable of binding 14 p.p.m. SO₂ at a free SO₂ content of 50 p.p.m. Much more information is required on the pyruvic and *α*-ketoglutaric acid content of ciders, particularly on how these keto-acids can be kept to a minimum in order to reduce the SO₂-binding power of ciders.

Xylosone in ciders is thought to arise from the dehydro-ascorbic acid present in the juice which undergoes hydrolysis and decarboxylation in the presence of SO₂ (7). The present calculations show that it can make an appreciable contribution to the SO₂-binding power of cider.

The four preceding carbonyl compounds occur in significant amounts only in ciders derived from sulphited fermentations. From the point of view of SO₂-binding power, the composition of a sulphited cider at the end of its fermentation is such that all the SO₂ added is combined, probably as acetaldehyde-bisulphite, and there may be a small amount of free acetaldehyde, as suggested by Paul (11). In this case the other carbonyl compounds present will exist entirely in the free state and, together with any free acetaldehyde, will be available to react with any further SO₂ that may be added. It follows, therefore, that the amount of SO₂ added to a juice should be the minimum required to give adequate control of the natural microflora, so that the bound SO₂ (as acetaldehyde-bisulphite) in the cider is kept as small as possible. The amounts of keto-acids and xylosone in cider, however, are not necessarily proportional to the amount of SO₂ added to the juice ; the factors influencing the production of these compounds and their metabolism by yeasts and bacteria are still under investigation.

Other Carbonyl Compounds. The remaining carbonyl compounds in Table I do not appear able to make a large contribution to the SO₂-binding power of cider. The concentration range given for the mono- and trigalacturonic acids is based on the total pectin content of the original juice. How far the polygalacturonide chains are degraded will depend on the conditions of fermentation and the pectic enzymes of the juice and yeast (12), but the concentration range quoted for the galacturonic acids, and therefore their SO₂-binding power, is probably a conservative estimate. The unfermentable sugar, xylose, is always present in sulphited ciders to the extent of about 0.05% and has only a small SO₂-binding power. Glucose is likely to be present in commercial ciders only as a result of sweetening the final product for sale ; the figure of 1.8% glucose is the amount that would arise by inversion of 3.4% sucrose (approximately 14 degrees S.G.). It should be pointed out, however, that the inversion of sucrose in cider is relatively slow. If, therefore, a cider is sulphited and bottled within a short time after sweetening, the amount of SO₂ bound to glucose will be small, at least for a few days, during which time the free SO₂ will be acting on any organisms picked up during the bottling process.

This investigation is continuing with the determination of the SO_2 -binding power of ciders, together with analyses for the more important carbonyl compounds. The number of ciders so far examined in detail is insufficient to draw any general conclusions except that the importance of pyruvic acid in sulphited ciders is clearly established. This work will be reported when more results are available.

Acknowledgments.

We gratefully acknowledge the assistance of Mr. R. A. Coggins in determining carbonyl compounds and of Mr. A. H. Sparks and Miss Valerie Fowler in determining dissociation constants.

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THE RAPID DETERMINATION OF IRON IN CIDER, PERRY AND FRUIT JUICES.

By C. F. TIMBERLAKE and GRACE M. HUNT

Introduction.

Excessive amounts of iron in cider can give rise to several undesirable features, and a rapid and simple method of iron determination would be of value, particularly to the small cider-maker with limited laboratory facilities. We have therefore investigated the possibility of determining iron directly in cider, without the preliminary destruction of organic matter which is the usual procedure when cider or other foodstuffs are analysed for trace metals. Banick and Smith (1) have already described a method for the "in situ" determination of iron in wines using 4,7-diphenyl-1,10-phenanthroline ("Bathophenanthroline"). We also have employed this reagent, which is very sensitive and specific for iron under certain conditions. Its red ferrous complex is readily soluble in organic solvents and so can be examined without interference from the background colour of the cider or fruit juice. Bathophenanthroline has also been used to determine iron in water (2), serum (3), urine (4,5), bismuth (6), culture medium (7), plant tissue (8) and petroleum oils (9), after previous destruction of organic matter, where present, usually by wet ashing.

In this paper, a rapid method of determining iron within the range 0–10 p.p.m. and requiring only 1 ml. of product is described, and the results obtained for some ciders, perries and fruit juices are compared with those obtained after the usual wet ashing of these products. The development of the method and studies of interferences due to fruit components are described.

Experimental.

METHOD.

Reagents

Water : glass-distilled.

4,7-Diphenyl-1,10-phenanthroline (Batho-phenanthroline) : 0.001M. Dissolve 0.033 g. in absolute alcohol with gentle warming and dilute to 100 ml. with absolute alcohol.

Hydroxyammonium chloride (A.R.) : 10% aqueous solution.

Acetic acid-sodium acetate buffer solution : M, pH 4.0. Mix 160 ml. of M acetic acid (29 ml. glacial acetic acid A.R. diluted with water to 500 ml.) and 40 ml. of M sodium acetate (13.6 g. $\text{CH}_3\text{COONa} \cdot 3\text{H}_2\text{O}$ A.R. diluted with water to 100 ml.).

Iron standard solutions.

Solution A, 1 ml. $\equiv$ 200 μg Fe : Dissolve 0.3454 g. ferric alum $\text{Fe}(\text{NH}_4)(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$ in 0.1 N hydrochloric acid and dilute with 0.1N hydrochloric acid to 200 ml.

Solution B, 1 ml. $\equiv$ 10 μg . Fe : Dilute 5.0 ml. solution A with 0.1N hydrochloric acid to 100 ml.

Chloroform : Pure or B.P. Grade.

Procedure.

In a clean, dry, stoppered test tube (Quickfit MF 24/2/6 B, 19, 150 mm. long test tubes were used) place the following reagents in the following order.

2.5 ml. water.

1.0 ml. cider (or other liquid product).

0.5 ml. 10% hydroxyammonium chloride.

2.5 ml. 4,7-Diphenyl-1,10-phenanthroline : 0.001 M.

1.0 ml. acetic acid-sodium acetate buffer ; M, pH 4.0.

Mix well after each addition. Stopper the test tube lightly, so that the stopper can still be moved, and place in a beaker containing water at 70°C, the level of which should be no higher than the level of the liquid in the test tube. Maintain at 70°C for 15 minutes. Cool to room temperature. Add 4.0 ml. chloroform (from a burette) and shake for 30 seconds. If a spectrophotometer is not available, allow the phases to separate and compare the colour of the lower phase with the colours produced by standard amounts of iron as described below. If a spectrophotometer is available, pour the contents of the test tube into a clean dry separating funnel and allow the phases to separate. Insert a plug of cotton wool into the short stem of the separating funnel and allow the lower coloured phase to run into a cell. Measure the extinction at 533 m μ using a 1 cm. cell (Unicam S.P. 500) or with green filter (Ilford No. 604) using a 0.5 cm. cell (Spekker absorptiometer), using chloroform as reference solution. Subtract the reagent blank, obtained by replacing 1.0 ml. cider by 1.0 ml. water. If the cider contains more than 10 p.p.m. iron, replace 1.0 ml. cider by 0.5 ml. cider and 0.5 ml. water.

Preparation of Standard Colours.

Carry out the procedure as described above except replace 1.0 ml. cider or related product by x ml. of iron standard (Solution B, where $x=0.1, 0.2 \dots 1.0$ ml.) and $(1-x)$ ml. water.

Results.

The iron contents of some ciders, perries and fruit juices were determined by the above method and the results were compared with those obtained after wet-ashing. Iron in the wet-ashed solutions was determined using (a) bathophenanthroline, with aliquots of the wet-ashed solutions treated at pH 4 as described under Method, with and without the heat treatment, and (b) 2,2'-dipyridyl (10) with the heat treatment recommended by Mason (11) to decompose any non-ionised iron complexes which may be present.

Ciders and Perries (Table I).

Ciders A-D were commercial products, while the remaining ciders and perries were small-scale experimental samples made at Long Ashton. The iron contents of the ciders and low-tannin perries found by the rapid method were within 0.2 p.p.m. of those found after wet-ashing. The values found for high tannin perries, which are particularly rich in leucoanthocyanins (12) were about 0.6 p.p.m. lower by the rapid method than after wet-ashing. Values found by the rapid method were not increased by increase of the bathophenanthroline concentration to $2.5 \times 10^{-3}M$. Omission of hydroxyammonium chloride or the heating procedure gave low values, but omission of the buffer had little effect. Recoveries of iron (5 p.p.m. Fe^{3+}) added to ciders A-D were 98, 95, 91 and 98% respectively.

TABLE I.
DETERMINATION OF IRON IN CIDER AND PERRIES.

Product	"Tannin" %	Iron content (p.p.m.)					
		Bathophenanthroline				Dipyridyl	
		Not ashed		Wet ashed		Wet ashed	
		Mean	Average deviation	Mean	Average deviation	Mean	Average deviation
Cider A	0.08	4.96	0.12	5.11	0.10	5.22	0.07
Cider B	0.10	7.36	0.07	7.47	0.13	7.48	0.02
Cider C	—	2.80	0.07	2.93	0.13	2.97	0.03
Cider D	0.16	5.36	0.05	5.52	0.11	5.54	0.01
Brown Thorne Cider	0.21	0.46	0.00	0.66	0.03	0.73	0.03
Pennard Bitter Cider	0.65	1.15	0.04	1.34	0.03	1.46	0.06
Hendre Huffcap Perry	0.05	0.76	0.05	0.93	0.02	0.94	0.00
Moorcroft Perry ..	0.25	1.00	0.03	1.25	0.01	1.34	0.01
Rock Perry A ..	0.78	1.01	0.08	1.60	0.02	1.68	0.05
Rock Perry B ..	1.04	1.26	0.08	1.86	0.08	1.95	0.05

TABLE II.
DETERMINATION OF IRON IN FRUIT JUICES.

Product	Iron content (p.p.m.)			
	Bathophenanthroline		Dipyridyl	
	Not ashed		Wet ashed	
	Mean	Average deviation	Mean	Average deviation
Black currant juice	11.5	0.4	11.7	0.4
Strawberry juice	0.89	0.11	1.20	0.11
Raspberry juice	2.15	0.02	2.24	0.08
Clear apple juice	0.46	0.02	0.57	0.08
Cloudy apple juice	0.59	0.00	0.80	0.00

Fruit Juices (Table II).

Iron contents found by the rapid method were within 0.3 p.p.m. of those found after wet-ashing of the juices. They were not increased by the use of stronger bathophenanthroline ($5 \times 10^{-3}M$). Recoveries of added iron (5 p.p.m. Fe^{3+}) were 95–100%, except with strawberry and raspberry juices (85%).

Statistical examination of the results (Tables I and II) indicated that the values found by the rapid method were significantly lower (1% level) than those found after wet-ashing. There was no significant difference in the values found for the wet-ashed samples, whether bathophenanthroline, with and without heat treatment, or dipyridyl was used.

Choice of Solvent and Method.

Solvents previously used to extract the ferrous bathophenanthroline complex from the aqueous phase have included *iso*-amyl alcohol (1.4, 9), *n*-hexyl alcohol (6,7), nitrobenzene (5) and chloroform containing 10% *iso*-amyl alcohol (8), and the extracts have been diluted to known volumes. In our preliminary tests, *iso*-amyl alcohol, chloroform and chloroform containing 10% *iso*-amyl alcohol appeared equally effective, whether single or double extractions were made. For each solvent, the molecular extinction coefficient of ferrous bathophenanthroline at 533 $m\mu$ was found to be within 2% of the published value of 22,400 (2).

We have used chloroform because of its high density, and measured the extinction of the organic phase after a single extraction and without further dilution. This method gave maximum sensitivity and reproducible results, provided that the volumes of water, alcohol and chloroform given under Method were strictly adhered to, since any alterations affected the volume, and hence the extinction of the organic phase obtained after shaking. Thus, when 0.25 ml. and 0.50 ml. of water used under Method were replaced by 0.25 and 0.50 ml. of absolute alcohol, the extinction due to 5 μg iron was reduced by 5% and 10% respectively. The findings indicate that iron contents 5% and 10% lower than their true values would be obtained if the product analysed (1 ml.) contained 25% v/v and 50% v/v respectively of alcohol. At the smaller alcohol levels in cider and perry, the errors expected due to this source would be only 1 or 2%.

A further possible source of error is the extraction of some of the natural coloured matter of the cider or related product into the organic phase. In the products analysed this was found to be negligibly small, but can readily be allowed for, if necessary, by using as a control 2.5 ml. absolute alcohol instead of the bathophenanthroline solution given under Method. The concentration of alcohol (33.3%) used in the rapid method was chosen to ensure that the bathophenanthroline, which is expensive and water-insoluble, remained and was effective in solution. It was essential that the colour be fully developed before the addition of chloroform, otherwise low results were obtained. In this connection, studies were made of possible interference by both inorganic and organic components of cider and related products.

Studies of Interference.

The substances examined were tested in acetate buffer as described under Method unless otherwise stated, with the replacement of the 2.5 ml. water and 1.0 ml. cider by 3.5 ml. aqueous solution containing the component and iron standard. The concentrations of component and iron refer to those in 1 ml. Iron was added in the ferric state (solution B) unless otherwise stated.

Inorganic Components.

Zinc and manganese (10 p.p.m.) lead, aluminium, nickel, tin and bismuth (5 p.p.m.), and orthophosphate (100 p.p.m.) did not interfere at an iron level of 10 p.p.m. At the same iron level, pyrophosphate (10 p.p.m.) reduced the extinction by 20% when the heat treatment was omitted but did not interfere when heating was carried out (cf. 7,14). Copper (10 p.p.m.) did not interfere at pH 4 but gave an increased extinction (5%) at pH 5.1 (cf. 4). Contrary to some workers (8), but in agreement with others (2, 9) we found no interference by perchlorate remaining in the wet-ashed solutions. Although turbidities sometimes developed when these solutions were heated, the chloroform extracts invariably remained clear (cf. 5).

Organic Components.

Fructose (10%), glucose (1%), sucrose (2%), sorbitol (2%), proline (0.1%), aspartic acid (0.1%), lactate (0.9%) and tartrate (1%) did not interfere. In the presence of malate (13.4%), colour development was slow, but practically complete after 15 minutes at room temperature. In the presence of citrate (4.2%), colour development was slow at room temperature (60% of the maximum after 15 minutes) but complete after heating at 70°C for fifteen minutes (cf. 6). Special attention was paid to phenolic compounds.

Phenolic Compounds.

Added ferric iron (5 p.p.m.) was recovered completely from solutions containing d-catechin (0.2 and 1%), chlorogenic acid (0.2 and 1%) and phloridzin (0.2%), but to the extent of only 76–81% in the presence of catechol (0.28–1.1%), both with and without heat treatment; recovery was complete, however, in the absence of hydroxyammonium chloride. Iron added in the ferrous state could be completely recovered both in the presence and absence of hydroxyammonium chloride.

Samples of mixed apple phenolics extracted from Tardive Forestier and young Dabinett fruit (15) and apple and pear leucoanthocyanins (13) were then examined. Percentage recoveries of added iron (5 p.p.m.) were as follows :—

	<i>Tannin</i> (per cent)	<i>Ferric Iron</i> NH ₂ OH.HCl		<i>Ferrous Iron</i> NH ₂ OH.Cl	
		absent	present	absent	present
Tardive Forestier phenolics	0.1		88		
	0.6		60		
Dabinett phenolics	0.12		82		
	0.6	95	37	95	35
Apple leucoanthocyanin	0.08	95	90		
Pear leucoanthocyanin	0.20		94	100	90
	0.38		89		

In the presence of hydroxyammonium chloride recoveries were low, whether iron was added in the ferric or the ferrous state. At the high level (0.6%) of Dabinett phenolics the red colour of the ferrous bathophenanthroline complex was evident in the cold (92% recovery) but faded on heating (37% recovery). Without hydroxyammonium chloride the recoveries were much higher. The findings suggest that any ferrous leucoanthocyanin complexes formed are not more stable than the ferrous bathophenanthroline complex. Although, as described earlier, the iron contents of ciders were usually low unless hydroxyammonium chloride was present, the opposite effect was found in the model systems. Since the low recoveries were associated with the presence of hydroxyammonium chloride, it was replaced by catechol and ascorbic acid as reducing agents in some further tests.

As previously stated, added ferric iron could be recovered fully from solutions containing catechol when hydroxyammonium chloride was omitted. In the presence of malate (1%), however, recovery was low (72%) at a catechol concentration of 0.28%. It was evident that a large excess of catechol would be required in the presence of other substances capable of forming ferric complexes, and when 0.5 ml. of 20% catechol was used (i.e. 10% catechol) instead of hydroxyammonium chloride, added iron was recovered to the extent of 96% in the presence of 1% malate pH 4. However, low results were obtained (60–70%) when this amount of catechol was added to ciders of known iron content.

Although ascorbic acid (0.5 ml. of 10% solution) gave good recoveries of added ferric iron when added instead of hydroxyammonium chloride to solutions containing catechol and Tardive Forestier phenolics, unsatisfactory results were obtained when it was added to ciders of known iron content.

Nylon Treatment.

Since low recoveries of iron were caused by leucoanthocyanins, the effect of Nylon, known to remove anthocyanogens (leucoanthocyanins) from beer (16, 17) was tested. Nylon was available as a powder (Nylon 66, I.C.I. Batch No. DS 3276) and paste (DS 3850) containing 81.5% moisture. Solutions of phenolics and perries were shaken with the powder at room temperature for 40 minutes or shaken with paste for 15 minutes and centrifuged. The paste was considerably more effective in adsorbing phenolic material than the powder.

Nylon powder (10% w/v) treatment reduced the "tannin" content of a solution of Dabinett phenolics from 0.58% to 0.08% and excellent recoveries of iron, added after Nylon treatment, were now obtained. Similar results were obtained with paste, as follows :—

Nylon Paste %	..	0	2	5	10	20
"Tannin" %		0.58	0.42	0.30	0.16	0.09
Recovery of iron %		37	46	60	82	96

The interfering phenolics removed by Nylon were shown by paper chromatographic examination to be mainly of leucoanthocyanin nature. However, when ferric iron was added to a solution of Dabinett phenolics in 0.1 M acetate buffer pH 4, before Nylon treatment, the amounts recovered were smaller—27% recovery with 10% Nylon paste and only 7% recovery with 20% Nylon paste. Recoveries from the acetate buffer in the absence of the phenolics were 99% and 85% respectively. Iron as well as leucoanthocyanins appeared to be removed by the Nylon, presumably in the form of an iron leucoanthocyanin complex.

Nylon-treated perries contained less iron, as determined by the rapid method, than untreated perries, although their "tannin" contents were reduced.

Perry Rock A. The iron contents with and without Nylon powder (10% w/v) and other treatments were as follows :—

Hydroxy-ammonium chloride	Nylon	"Tannin" %	Iron content (p.p.m.)	
			Room temp. 15 min.	70°C 15 min.
Absent	Absent	0.78	0.19	0.80
Present	Absent	0.78	0.55	1.01 (mean)
Absent	Present	0.45	0.10	0.50
Present	Present	0.45	0.35	0.70

The highest value was obtained on heating in the presence of hydroxy-ammonium chloride in the usual way and without Nylon. Although small amounts of Nylon paste (up to 10% w/v) had little effect, larger amounts also reduced the iron content of perry Rock A, as follows :—

Nylon Paste %	10	25	50
"Tannin" %	0.26	0.10	0.04
Iron content (p.p.m.)	1.02	0.79	0.67

Moorcroft perry. Nylon powder treatment (10% w/v) of Moorcroft perry reduced its iron content from 1.00 p.p.m. (Table I) to 0.44 p.p.m. and its "tannin" content from 0.25% to 0.04%. Recoveries of iron added to the untreated perry and to the perry after and before nylon treatment were 92%, 102% and 34% respectively.

The results suggest that iron in perries and ciders is partly combined with leucoanthocyanins and that the iron so combined can be removed to some extent with Nylon.

Summary.

A rapid method of determining iron in ciders, perries and fruit juices, without preliminary destruction of organic matter is described. The iron contents of the ciders, low-tannin perries and fruit juices analysed were not more than 0.3 p.p.m. lower than those obtained after wet-ashing; greater differences in iron content were found with high-tannin perries. Tests with fruit components indicated that an interaction between iron, leucoanthocyanins and hydroxyammonium chloride was partly responsible for the lower values obtained when the wet-ashing procedure was omitted. Nylon treatment of perries to remove leucoanthocyanins also removed some iron, as determined by the rapid method, possibly in the form of iron leucoanthocyanin complexes.

Acknowledgments.

We wish to thank Miss M. E. Holgate for statistical examination of the data, Mr. A. H. Williams for a supply of apple leucoanthocyanin, Miss V. Fowler for determining "tannin" contents and Mr. J. H. Llewellyn for the perries.

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A COMPARISON OF SOME VARIETIES OF VEGETABLES PRESERVED BY CANNING AND BY FREEZING ; PROGRESS REPORT VII.

By ALICE CRANG (the late) and MARGARET LEACH.

The quality assessment of varieties of beans, peas and sweet corn that have been tested for three or more years is reported. Average markings for colour, texture, flavour and percentage waste in preparation are given.

Experimental.

Vegetable used. All vegetables were grown at Long Ashton, picked when at their prime and preserved without delay.

Preservation methods. The methods of canning and freezing beans and peas were the same as those used previously (1). The sweet corn was preserved in two forms : whole grain and cream style. For whole grain, sheath and silk were removed, the cobs were scalded in boiling water and the grains were then cut off the cobs. For deep freezing, the cooled corn was packed into airtight containers, frozen* and stored in a deep-freeze cabinet (-10° to 0°F). For canning, the corn was heated to boiling point in 2% brine and packed into 1 lb. SR cans, using 10 oz. grain to 5 fl. oz. brine, sealed*, and processed at 10 lb. pressure for 60 minutes ; it was then stored at room temperature.

For cream style, the grains were cut off the cobs, which were then scraped. The corn and scrapings were cooked for $4\frac{1}{2}$ minutes in a little water to prevent burning. For deep freezing, the creamed corn was cooled and frozen* as for whole grain. For canning, 1 lb. SR cans were filled with 1 lb. creamed corn, sealed* and processed at 10 lb. pressure for 85 minutes ; they were stored at room temperature.

The vegetables were stored for four to five months before tasting.

Judging. A maximum of 5 marks each was allotted for colour, texture and flavour (2). The tasting panel consisted of 12-15 members of staff, many of whom have been judging regularly in similar trials for several years.

Results.

Beans, Broad.

Seven varieties were frozen in 1959 and eight in 1960 ; all gave satisfactory results. Because of previous experience (2), no canning was undertaken.

The average markings for the varieties Aquadulce Longpod, Leviathan and White Fan, all tested for 3 years, are given in Table I.

Beans, French.

Seven varieties, 3 climbing and 4 dwarf, were tested both by canning and freezing. All varieties of climbing bean required stringing, and all varieties of dwarf bean were tested as "stringless", having only the tips removed in preparation. (The varieties Golden Butter and Wax Pod were found to need stringing if picked at maturity). The dwarf beans were cut in half lengthways and the climbing beans were cut into thin slices.

* For details of processing see *M.A.F.F. Bulletin No. 21*. H.M.S.O., 9th Edn., 1958.

The scores for the seven varieties tested for 3 seasons are given in Table 1.

TABLE I.
MEAN MARKS FOR CANNED AND FROZEN BEANS.
(Maximum 5).

Variety	Colour		Texture		Flavour		Waste	
	C	F	C	F	C	F	% Total Weight	
							Mean	Range
<i>Broad</i>								
Aquadulce Longpod	—	3.2	—	2.9	—	2.8	69	68-70
Giant Windsor	—	3.0	—	2.5	—	2.5	66	60-76
Leviathan	—	3.5	—	3.0	—	3.3	68	66-70
White Fan	—	3.2	—	2.7	—	2.7	57	51-65
Mean 8 varieties 1960 ..	—	3.5	—	3.2	—	3.1	71	65-79
„ 7 „ 1959 ..	—	3.1	—	2.9	—	2.9	67	62-77
„ 10 „ 1958 ..	—	3.4	—	3.1	—	3.2	67	56-74
<i>French Dwarf</i>								
Lightning	2.4	3.3	2.8	2.6	2.3	2.7	24	20-26
Golden Butter (stringless) ..	0.5*	1.9	2.5*	2.7	1.6*	2.5	9	7-12
Mont d'Or	1.1	1.8	2.6	2.8	1.8	2.6	12	11-14
Phenix Claudia	1.2	3.3	2.7	3.2	1.8	2.9	12	10-13
Tender Green	1.9	3.6	3.2	3.4	2.4	3.0	10	9-12
Wax Pod	0.6	2.1	2.0	2.7	1.5	2.0	9*	7-11
<i>French Climbing</i>								
Tender and True	2.4	4.1	2.5	2.8	2.1	2.7	23	23-24
Mean 7 varieties 1960 ..	1.8	3.2	3.3	3.0	2.4	2.7	—	—
Stringless	—	—	—	—	—	—	9	8-10
Others	—	—	—	—	—	—	18	14-21
Mean 12 varieties 1959 ..	1.7	3.2	2.6	2.9	1.8	2.6	—	—
Stringless	—	—	—	—	—	—	11	9-14
Others	—	—	—	—	—	—	22	20-24
Mean 17 varieties 1958 ..	1.7	3.0	2.7	3.2	2.1	2.8	—	—
Stringless	—	—	—	—	—	—	12	9-14
Others	—	—	—	—	—	—	26	22-31

C=Canned ; F=Frozen ; *Two years only.

As in previous years, the frozen beans were preferred to the canned ones for colour and flavour. The colour rating of golden varieties was lower than for green varieties in both canned and frozen packs.

Colour marking for golden and green beans tested for 3 years.

		Canned	Frozen
Mean 3 varieties	Golden	0.7	1.9
Mean 4 varieties	Green	2.0	3.6

The dwarf variety Lightning gave satisfactory results both canned and frozen, as did the climbing variety Tender and True. For freezing, the dwarf varieties Tender Green and Phenix Claudia were good.

Peas.

Six varieties were tested in 1959 and six in 1960. The six varieties tested for 3 years are reported in Table II. In the canning trials, Peter Pan was fair, but the other 5 varieties were weak in colour; Sutton's Pioneer and Poulwell were also weak in flavour. Much better results were obtained from frozen packs: all the varieties scored good ratings, especially Daffodil, Early June and Sutton's Show Perfection; the results from these three varieties are comparable to those obtained with Onward and Thomas Laxton (1). The waste on preparation (61%, 58% and 56%) was less than with these two varieties (75% and 72%).

Sweet Corn.

Four varieties have been tested for 3 years; the results are given in Table II.

TABLE II.
MEAN MARKS FOR CANNED AND FROZEN PEAS AND SWEET CORN.
(Maximum 5).

Variety	Colour		Texture		Flavour		Waste	
	C.	F.	C.	F.	C.	F.	% Total Weight	
							Mean	Range
<i>Peas</i>								
Daffodil	1.8	4.1	2.2	3.3	2.2	3.2	61	57-65
Early June	1.8	4.1	2.2	3.5	2.4	3.5	58	56-61
Peter Pan	2.1	3.8	2.5	3.3	2.6	3.4	56	47-62
Poulwell	1.8	3.9	2.0	2.9	1.4	2.8	56	53-61
Sutton's Pioneer	1.9	3.8	1.8	2.9	1.8	3.0	57	50-73
Sutton's Show Perfection	1.6	4.1	2.2	3.5	2.1	3.2	56	53-58
Mean 6 varieties 1960.. ..	2.3	3.9	2.7	3.3	2.3	3.4	63	53-75
Mean 6 " 1959:	1.9	4.0	2.1	3.3	2.3	3.2	54	50-60
Mean 8 " 1958.. ..	1.9	4.1	2.1	3.3	2.2	3.2	60	55-65
<i>Sweet Corn</i>								
Canada Cross								
whole grain }	2.6	4.0	2.2	2.5	2.5	3.0	{ s 28*	28-29
cream style }	2.6	3.1	2.1	2.6	2.2	2.6	{ c 31*	29-33
Early Golden Market								
whole grain }	2.4*	3.1	2.5*	2.3	2.6*	2.6	{ s 35*	30-41
cream style }	2.0*	2.4	2.7*	2.1	3.4*	2.3	{ c 31*	30-31
Golden Bantam								
whole grain }	2.5	3.9	2.7	2.9	2.5	3.4	{ s 38*	37-38
cream style }	3.0	4.1	3.1	2.6	3.1	2.7	{ c 30*	28-32
John Innes								
whole grain }	2.4	3.7	2.5	2.8	2.7	3.1	{ s 32	30-36
cream style }	2.5	3.2	2.3	2.8	2.9	2.9	{ c 30	26-33
Mean 2 varieties 1960								
whole grain }	2.4	4.2	2.2	2.7	2.9	2.8	{ s 30	29-30
cream style }	3.1	3.8	2.0	3.0	2.7	3.4	{ c 33	33
Mean 4 varieties 1959								
whole grain }	3.0	3.7	2.5	2.8	2.9	3.3	{ s 32	28-38
cream style }	3.0	3.2	2.6	2.4	3.1	2.7	{ c 28	26-30
Mean 3 varieties 1958								
whole grain }	2.2	3.8	2.6	2.7	2.3	3.2	{ s 38	35-40
cream style }	2.0	3.4	2.6	2.5	3.0	2.0	{ c 31	29-32

C=Canned; F=Frozen; s=Sheath; c=Cob; *Two years only.

The canned corn, either whole grain or cream style, retained its colour and flavour satisfactorily, although the corresponding frozen product obtained a higher rating in most cases. Golden Bantam gave good results in all processes.

The waste on the cob was approximately 30% for all varieties reported, but the waste from the sheath leaves varied from a mean of 28% for Canada Cross to 38% for Golden Bantam, both losses being expressed as a percentage of the original weight of sheathed cob.

Summary.

The report gives the quality assessment and wastage during preparation of five varieties of broad bean preserved by freezing, and seven varieties of French bean, six varieties of pea and four varieties of sweet corn preserved by canning and freezing.

Acknowledgments.

We wish to thank Miss P. Davey for help in preserving the vegetables, and the tasting panel for their continued assistance.

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BASKET WILLOW GROWING IN NORTHERN EUROPE.

By K. G. STOTT.

An economic review of the British basket willow growing industry (1), made in 1960 to supplement an earlier account of methods of cultivation and processing (2), showed that the decline of the industry was due to its inability to meet the increased cost of labour, and to competition from cheap imported willows and baskets. Many of these originated in Poland, Yugoslavia and Bulgaria, and it appeared that whilst willow growing was declining in Western Europe, both production and research were expanding in Eastern Europe. Hence in the autumn of 1960 growers and research institutes were visited in Poland and East Germany to discover whether this expansion had a political foundation or was due to advances in methods of cultivation, pest and disease control that might be applied in Britain.

West Germany and Holland were also visited to study research and methods of production, and to obtain a more comprehensive view of the factors causing a decline of willow growing in Western Europe. This report compares the organization, methods and economics of willow growing in Britain with those in the countries visited.

Organization.

In Communist regimes all the means of production are state-controlled : more strictly in East Germany, where all land is collectivized, than in Poland, where only 30% is collectivized. Fifty per cent of Poland's 10,000 acres of willows are controlled and marketed by a regional agency of the special government department dealing with willows (L.A.S.). The remainder are owned by peasants, village communes, collective and co-operative farms and by three of the five state companies that control all processing and basket making. All exports of willows and baskets are handled by the state agency, P.A.G.E.D., which markets forest products.

In East Germany the government has stimulated demand for willows and baskets by a decree that baskets be used by industry and agriculture in preference to other containers. Since most established willow beds were lost in partition, or ceded to Poland, the central state planning authority directed that 10,000 acres of willows be planted to meet this demand. The State Forest Service was made responsible for willow growing, processing and basket making and, so far, 5,000 acres have been successfully established.

In contrast there is no state control in West Germany, Holland and Britain : willow growing, processing, marketing and basket-making are in the hands of individuals, many of whom make a living from about 10 acres of willows. In Holland, the acreage grown by individual owners tends to be larger, especially in the deltas where willow sticks are grown, and in the very heavy clay soils of the Lek (Rhine) valley where large estates are farmed extensively.

In Holland, Britain and East Germany there is an officer, employed by the state and resident at a state research station, responsible for advice and research on basket willows ; in Poland the officer is attached to the government department. There is no state willow officer in West Germany ; some provinces

like Bavaria and Ober Franken provide their own, but there are a few specialists, with interest in some particular aspect of willow culture, distributed throughout the country at various state research stations.

Basket Willow Growing.

Area. It will be seen from Table I that the acreage of basket willows in Britain is small compared with that in other European countries.

TABLE I.
EUROPEAN ACREAGE OF BASKET WILLOWS (1960).

Poland	..	..	10,500
Holland	..	..	10,000
West Germany	..	..	7,500
East Germany	..	..	5,000
Britain	..	..	1,784

In Britain and Holland willow growing is largely restricted to one or two localities. Over three-quarters of Britain's willows are grown in the Langport district of Somerset (1), and in Holland a similar proportion is grown in the deltas and on land outside the flood walls. The other important area of Dutch production is centred on Langbroek in the Lek valley and owes its importance to the extremely heavy nature of the soil and to the extensive system of land management. Table II shows that willow growing is widely distributed throughout the provinces of West Germany (3); the most important areas are the valleys of the Elbe, Weser, Main, Danube, Rhine and Ruhr.

TABLE II.
BASKET WILLOW ACREAGE IN THE PROVINCES OF W. GERMANY (1956).

Schleswig-Holstein	..	..	..	1,838
Hamburg-Bremen	..	..	..	207
Lower Saxony	..	..	..	1,892
North Rhine—Westphalia	..	..	..	1,887
Hesse	..	..	..	287
Rhineland Palatinate	..	..	..	1,062
Baden-Württemberg	..	..	..	388
Bavaria	..	..	..	874
Total	..	..	..	8,435

Similarly, willows are grown in all parts of East Germany and Poland, but usually in large blocks of 50-100 acres instead of the small fields of 3-5 acres found in West Europe. Brzeg in Silesia still remains the largest single willow growing area in Europe; before the war one holding alone exceeded 2,500 acres and employed 100 workers.

Soils. In Poland willows are grown on medium to very poor sands in valleys or near lakes, where there is a risk of summer flooding. For the greater part of the year the water-table lies three feet below the surface and may drop to five feet.

In East Germany the areas allotted to the State Forest Service are restricted to soils and situations too poor for agriculture; consequently only difficult soils are available for willow growing. Most willows are grown on sands, similar to but slightly more fertile than those in Poland, but some have been established on the peaty fen soils overlying sand in the northwest of the country.

Many of these contain 80% organic matter and have a pH ranging from 3 to 6 and a water-table often less than 12" from the surface. In better parts, where the water-table is normally about 20" from the surface and the peat layer is less than 15" thick, the underlying sand can be ploughed up and mixed in to create a soil reasonably good for willow growing.

The same conditions are met in the Elbe district of West Germany : they are common to the belt of terminal moraines stretching from North Holland through Germany to Poland and Russia. In contrast, the soils of the Danube and Main valleys in the south are fertile and neutral, with a high proportion of silt and alluvium. The water-table in the Main valley is usually 24" below ground level, but the river can flood at any time ; hence the land is given to rich pasture or willow growing and is very reminiscent of the Somerset willow growing area in Britain.

The soils of Somerset are more fertile and heavier than any so far described ; many contain 60–80% clay. The content of organic matter is usually 10–20%, but some soils may contain 50% (4) : they are then similar to, but more fertile than the fen soils of East Germany. The water-table is often at the surface in winter and rarely falls below 18". Flooding is such a constant danger that a very comprehensive system of drainage ditches and pumping stations has to be maintained, but this allows great flexibility in the control of ground water, and in dry summers the ditches can be used for irrigation.

In Holland willows are grown on the alluvial soils of the delta, and inland around Langbroek, where the soil contains 80–90% clay, 5–10% sand and 2% organic matter. The area was reclaimed only in 1880 ; the water-table is now usually 10–15" below ground in winter, but falls to 3 ft. in summer. Even so, the soil is so heavy and sticky that the owners prefer to farm extensively, growing coppice crops of willow, ash, etc., and more recently top fruit.

Climate. In Holland and Britain the mild climate allows harvesting and processing to proceed throughout the winter without hindrance ; cut willows can be placed outside in pits, and begin to sprout in March ready for peeling into white. Establishment of a new bed is always easy and losses of cuttings are very few. At the other extreme, in Poland, the winters impede harvesting and processing and prevent the use of outside pits. In the spring the ground is cold and dry and the loss of cuttings during establishment can be very serious.

Varieties. A distinction can be drawn between Holland and Britain which have clay soils on which *Salix triandra* predominates, and Poland and East Germany with sandy soils on which *S. americana* predominates. Other species are grown for special purposes : in Britain *S. viminalis* is grown for sticks and in the Dutch deltas a total of 7,000 acres of this species and of *S. dasyclados* is grown on a three-year rotation for sticks and barrel staves ; *S. americana* is grown only on the poor sandy soils of Limburg in the south ; elsewhere it grows too vigorously, producing brittle rods with a high proportion of pith. In Poland, *S. americana* produces only light crops, hence varieties and hybrids of *S. viminalis* and *S. purpurea* are grown for more robust material. In East and West Germany these two species and their hybrids are more important.

Establishment. Cuttings 15" and 12" in length are used in Holland and Britain respectively. They are inserted into well-cultivated ground and failures are few, but in Poland and East Germany, where 10" cuttings are used, establishment is often difficult. Research is being conducted into the

optimum length of cutting and the proportion planted above and below ground; whether to plant singly, or in twos or threes as a safeguard against individual failures. In these two countries, each cutting usually produces one maiden shoot, 3-4 ft. in length, whereas in Holland and Britain three shoots of 3-5 ft. would be expected.

In all countries the cuttings are planted in rows about 24" apart, but within the row they are planted 4" apart in Poland, 8" apart in Germany, 14" in Britain and 20" in Holland. In the West, few alleyways or head-lands are left because the land is expensive and often surrounded by drainage ditches, whereas in East Germany generous allowances are made to facilitate the use of machinery.

About 2" of each cutting is left protruding out of the ground but, despite variation in harvesting methods, stools of *S. americana* are always lower and flatter than those of *S. triandra*, and these are lower than *S. viminalis* and the hybrids of *S. alba* sometimes grown in very wet sites in Poland and East Germany.

Rotation. The beds are retained for up to 15 years in Poland and East Germany, compared with about 20 years in West Germany and Holland. This would probably be the most economic rotation in Britain, but well-maintained beds often continue in profitable production for 50 years. In Britain economic yields are not expected until the third year, and peak production is not reached until the seventh year, but early yields are important in Poland and East Germany because the soils soon become exhausted. Research has shown that the improved yield in the second year well repays the extra cost of the cuttings and labour required for high-density planting.

In Poland *S. americana* produces high quality crops of slender rods rarely more than 5½ ft. high, and yields about 4½ tons of green rods per acre. Higher yields are obtained in East Germany, and as much as 7 to 8 tons per acre in West Germany, where this species often reaches 8 ft. in height. In Holland and Britain, *S. triandra* grows up to 9 ft. in height and yields 5 to 8 tons per acre; *S. viminalis* grows to 11 ft. in height and often yields over 10 tons per acre.

Weeds, Pests and Diseases. The poor sandy soils of Poland do not support vigorous weed growth, especially as *S. americana* casts a deep shade at the high planting-density employed; weeding is limited to light scuffling, or hoeing, usually with horse-drawn implements. In no country is weed growth important in crops of *S. viminalis*, *S. purpurea* or their hybrids.

S. triandra does not cover the ground as well as *S. americana* and it is only in Holland and Britain that grass growth is troublesome. It is usually cut by hand sickle or by small powered cutters, but more recently effective control has been obtained with dalapon applied in spring at the rate of 5 lb./100 gal./acre. Sometimes Dodder (*Cuscuta* spp.) damages beds in Poland and East Germany, but dicotyledonous weeds are unimportant compared with those, notably Bindweed (*Calystegia sepium*) and Cleavers (*Galium mollugo*) that flourish in the rich soils of Holland and Britain. Cleavers is a serious weed in Holland, and is now controlled by applications of tar oil in spring. It has never been important in British willow beds where tar oil spraying has been a common practice for a decade or more.

Pests and diseases vary with the willow species grown. Amongst insects, willow beetle (*Cryptorrhynchus lapathi*) is the most widespread and constitutes

PLATE V.

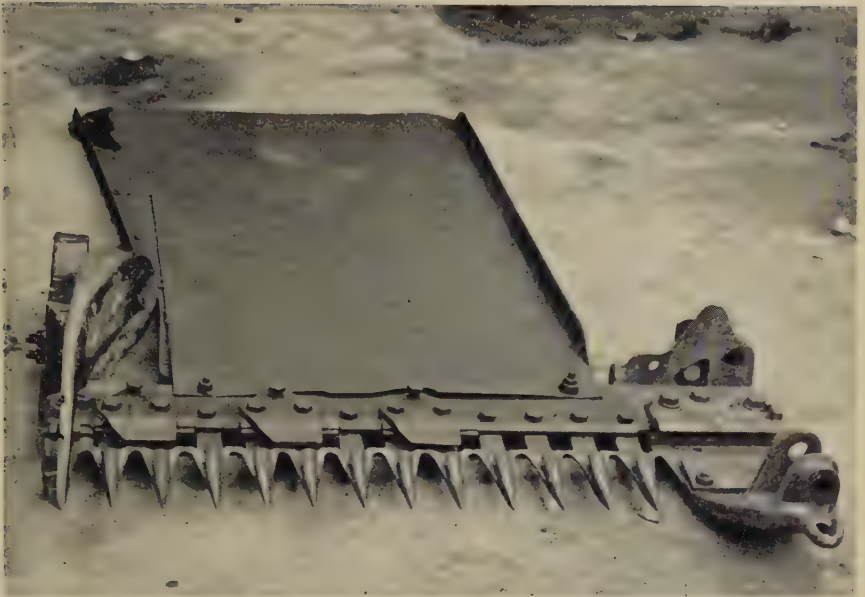


Fig. 1. Reciprocating tractor-mounted cutter bar and collecting tray used to harvest willows in Eastern Germany.



Fig. 2. West German willow stripping machine, showing the horizontal metal spirals which remove the bark.

PLATE VI.

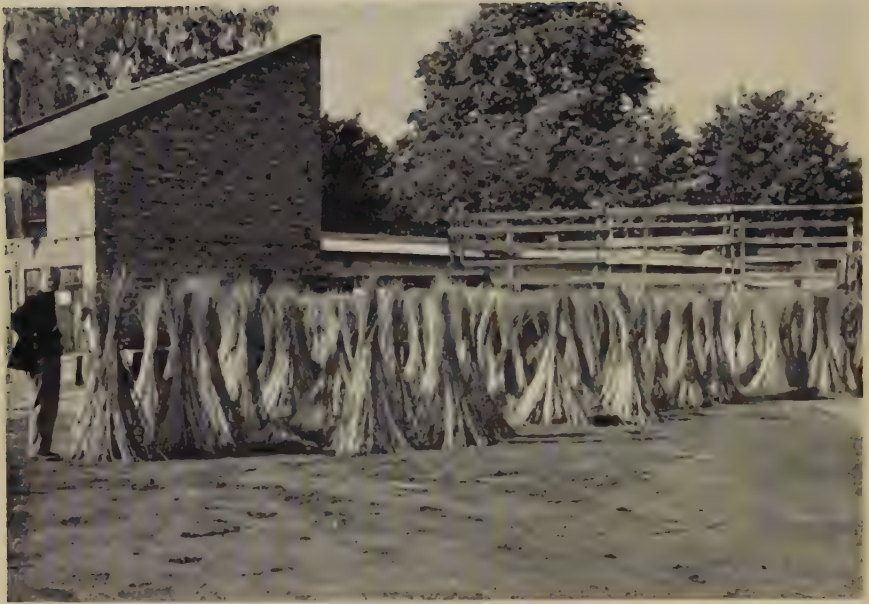


Fig. 3. Buff willows drying at a Polish willow-processing factory.



Fig. 4. The Town Council Chamber, Michelau, Western Germany. Basketware has been used for the ceiling, ventilators, ceiling and wall lamps, and chairs.

a major pest, particularly in those countries where *S. americana* predominates. Its life history is variable : it is reported that in Holland it spends its second winter as the pupa, whilst in Germany it spends it as the adult. Where it overwinters as the pupa, some measure of control can be effected by trimming the stools in early spring and burning the trimmings ; this method has been successfully applied in Hungary. No completely satisfactory chemical method of control has yet been found : of the materials tested, dieldrin appears to be the most promising. Whilst most other pests can now be controlled with modern insecticides, application is still a difficult problem ; hence in all countries trials are in progress with knapsack and other types of mist-blowers.

Fortunately, serious attacks of disease are infrequent in willows, though when they arise they are difficult to control. *S. americana* and hybrids of *S. viminalis* and *S. purpurea* suffer locally from dieback disease, caused by *Fusicladium saliciperduum* and *Phylospora miyabeana* ; these do not attack *S. triandra* appreciably : willow rust, *Melampsora amygdalinae*, which lowers the quality of the rods, is more important.

Harvesting and Processing. In Britain and Germany, the rods are harvested and processed by individual growers, sometimes with the help of casual labour. A large proportion of the Dutch one-year-old crop is sold standing ; the three-year-old sticks are cut and then converted into barrel staves by a travelling government workshop. In Poland, cutting is organized by the state agency (L.A.S.), after which the rods are graded and processed at a central depot of the local basket making and processing company. There are five companies covering Poland, each responsible to its provincial council of state, which controls the production of all commodities, sets quotas and targets and fixes prices, etc. Polish willows are usually cut by casual labour, often in gangs of over 100. The rate of pay is about £4 per week, equivalent to a third of the British rate.

On the Continent rods are sometimes cut singly by secateurs, but normally a sickle is used to cut a number of rods at one stroke, as in Britain. Attempts are being made to cut willows mechanically, particularly *S. americana*, whose stools always remain low and small. For the past three years in Limburg, Holland, cutting has been accomplished by a small hand-operated machine without loss in yield or quality. Trials are in progress in Britain and West Germany with adaptations of existing small cutting machines. Unfortunately there is little prospect of persuading manufacturers to produce a special machine because the numbers required would not pay for the cost of development. In Poland an advanced stage has been reached in the design of a large tractor-mounted cutter, working on a rotary principle, whilst in East Germany trials with a reciprocating tractor-mounted cutter-bar (Fig. 1.) were carried out last season without damaging the willow stools ; it is now planned to incorporate a device for collecting and tying the cut rods into bundles.

East German willows are used only for rough basketry, hence grading and processing are reduced to a minimum. Only a few are stripped for white, and even fewer buff are produced as, unlike *S. triandra* or *S. americana*, varieties and hybrids of *S. viminalis* and *S. purpurea* do not produce a rich brown colour.

In other countries the cut willows are graded into lengths, in units of one foot in Britain and 10 cm. on the Continent. They are then processed into buff or white, the latter being greatly preferred on the Continent. In Poland some willows are forced in heated cellars, or specially constructed pits under glass,

so that peeling can begin in February. Over 50% are still stripped by hand, mostly by casual labour. The stripping machine consists of two contra-rotating drums, carrying stripping breaks; in feeding the willows between the drums care is required lest the slender rods of *S. americana* be damaged. For the same reason the West Germans dislike the single drum machine used in Britain and prefer a machine in which the bark is removed by 6 to 8 loose metal spirals, 4" in diameter, which are mounted horizontally on the revolving drum (Fig. 2.)

The more progressive growers in West Germany and Britain use drying plant for the stripped willows, otherwise they spread them out along any convenient hedge or fence. In Poland the willows are dried in small conical stooks (Fig. 3.), and then graded into length and into three quality classes, the first quality being reserved for export. Before despatch their quality is re-checked by P.A.G.E.D. officials, to maintain the reputation for good and uniform quality that Polish willows enjoy in Western markets.

Basket Making.

About two-fifths of the Polish basket makers have been collected together into factories run by state basket making and processing companies. The smaller factories employ about 20 workers but the largest, at Brzeg, employs 200. In East Germany there are few, if any, private basket makers. They have been collected together into small groups and are employed by the State Forest Service mainly in making simple rough baskets for the home market. In contrast, Poland produces high quality basketware of every size and description, much of it specially for export.

There is no association of basket makers into factories in West Germany. Around Lichtenfels and Michelau, perhaps the most important basket-making centre in Europe, they work at home and their produce is collected and marketed by merchants: about 100 firms of merchants lie within a radius of 15 miles of Michelau. The first baskets were made in 1770, and by 1910 there were over 30,000 basket makers; there are now only 8,000, but this is more than twice the number employed in Britain. They specialize in skein work: each rod is split into two or three skeins which are then pared down to uniform width and thickness and used to make fancy baskets of all kinds—table baskets, handbags, lampshades, etc. (Fig. 4.).

Local skill is perpetuated by the German School of Basketry at Lichtenfels and preserved by the German Basket Museum nearby, which contains a collection of over 500 baskets. Opened in 1905, the school teaches, in a three-year residential course, all branches of the trade to students between the ages of 14 and 17. Formerly there were 60–70 students, but now there are only 20. The staff investigate methods of processing, bleaching and dyeing, and do much to promote basketry, notably by encouraging architects to incorporate basketry in the interior design of concert halls and other public buildings.

About three-quarters of Holland's willows are converted into barrel staves for export to Denmark. Most of the remainder are made into agricultural and fishing baskets, or into basket furniture; formerly 2,000 workers in the Langbroek area alone made nothing but bushel baskets for the British fruit trade.

There is no specialized basket-making area in Britain; individuals and institutions engaged in the trade are found all over the country. In East Anglia local supplies of *S. viminalis* are made into fishing and agricultural baskets,

but most of Somerset's production of *S. triandra* is made into domestic and trade baskets, and into large containers used by the Post Office, Railways, textile mills, etc. Little, if any, of the fine skein work characteristic of West Germany is made in Britain, even by individual craftsmen. About 35% of the willows used in Britain are converted into basketware in workshops for the blind and disabled (5).

Economics.

Willow growing is increasing in Poland and East Germany and declining in the other countries visited. These trends are due mainly to considerations of politics and labour relations, rather than to advances in technique. Willow growing and basket making require much labour and cannot be mechanized easily. They flourish only where labour is cheap and plentiful, as in Poland, which has a low standard of living and insufficient employment. Unlike Britain, there is no difficulty in obtaining casual agricultural labour, or apprentices for basket making. Whereas only 120 basket makers were registered in Poznan before 1939, there are now 8,000; the production of baskets has doubled since 1956. A fully trained basket maker is reputed to earn as much as a fully trained industrial worker, i.e. £300 per annum calculated at the tourist rate of 67 zlotys/£, and though the ideal of equal pay for equal skill may not be fully achieved, it is doubtful whether differentials are as great as they are in Western Europe. In Poland, willows are thus a remunerative crop that can be grown on poor sandy soil that would otherwise be of little value, and can be sold abroad for much-needed foreign currency.

In East Germany, the expansion of willow growing has a purely political foundation. Government has decreed that a certain acreage of willows is required within the next decade to meet the need for heavy industrial and agricultural basketware, and has made the Forest Service responsible for production. However, East Germany is very short of labour and, since the Forest Service find it difficult to obtain casual labour for weeding and harvesting, great efforts are being made to mechanize production. Moreover, since East Germany is not, like Poland, aiming to produce a high quality product for export, heavy-cropping varieties of inferior quality can justifiably be grown and baskets can be made of brown, unpeeled willow, thereby reducing processing to a minimum. Great basket-making skill is not essential, hence a quick turnover is possible and earnings can be relatively high. Few private basket makers exist; those employed by the Forest Units earn about £500 per annum, about half the amount earned by skilled workers in the motor industry or the mines.

In West Germany, Holland and Britain, willow-growing and basket-making are declining because of their inability to pay labour rates commensurate with those of industry, and to compete with cheap imports of willows and baskets, mainly from Poland, Yugoslavia and Bulgaria; the last country can produce baskets of excellent quality for half the cost in Germany. As the home production of baskets falls, so does the demand for willows. The demand for baskets is not static: the trend in modern packaging and marketing is towards non-returnable containers; already the demand for the bushel fruit baskets and butter-barrel staves once produced in Holland has almost disappeared. Plastic containers increasingly compete with baskets, and many German merchants prophesy that they will eventually replace the simpler kinds of basket.

However, it is not likely that willow growing and basket making will die out completely. In West Germany the merchants consider that there will always be a luxury demand for fine baskets regardless of the price, so that a small area of willows will always be needed. In Holland willows are used to stabilize the delta and river flood land, and to trap silt and build up solid ground. Around Langbroek they are grown because the soil is too heavy and sticky for other crops, and because the owners favour extensive farming which employs the minimum of labour and involves growing a high proportion of coppice crops, which need little attention in summer and can be sold standing in winter. More recently top-fruit growing has been introduced, often in combination with willow growing; and many farmers devote the summer and autumn to top-fruit growing and marketing and the winter to willow harvesting and marketing, the two occupations being reasonably complementary. There are thus good reasons for the continuation of willow growing in Holland, apart from the requirements for basket making.

Though the British willow growing industry suffers from high labour costs and faces severe competition from imports, these disadvantages are offset by climate, soil and situation, which in Somerset produce conditions ideal for willow growing; it seems likely that some demand for willows will always be forthcoming from institutions such as the Blind Asylums.

Summary.

The organization, methods and economics of willow-growing and basket-making in Poland, East and West Germany and Holland are compared with those in Britain.

Salix americana predominates on the poor sandy soils of Poland and East Germany, and *S. triandra* on the fertile clay soils of Holland and Britain. Weeds, pests and diseases are more important in the West than in Poland and East Germany, where more difficulty is experienced with establishment and processing.

Willow-growing and basket-making prosper in Poland because of the abundance of labour and in East Germany because of government direction; they are declining in the West because they cannot pay labour rates competitive with industry, but they are not likely to die out completely.

Acknowledgments.

I wish to record my appreciation of the kindness and co-operation shown by official and private growers in the countries visited and of a grant from the Agricultural Research Council towards travelling expenses.

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A NOTE ON WIND SPEED AND DIRECTION AT LONG ASHTON.

By G. E. CLOTHIER.

The Research Station is situated in a valley about 1,000 yards wide that runs approximately west and east. The floor of the valley is about 160' O.D. ; the hill to the north rises to over 400' O.D. and the nearest slope of the more undulating country to the south reaches 250' O.D.

Regular readings of wind strength and direction have been taken since the installation in 1947 of a Dines Pressure-tube Anemograph. Examination of the records for 1947-1960 shows little deviation from the pattern in 1956-1960. A wind rose has therefore been constructed (Fig. 1) for these five years to show the percentage frequencies of winds above and below a mean speed of 7 miles per hour from twelve points of the compass, and of calms.

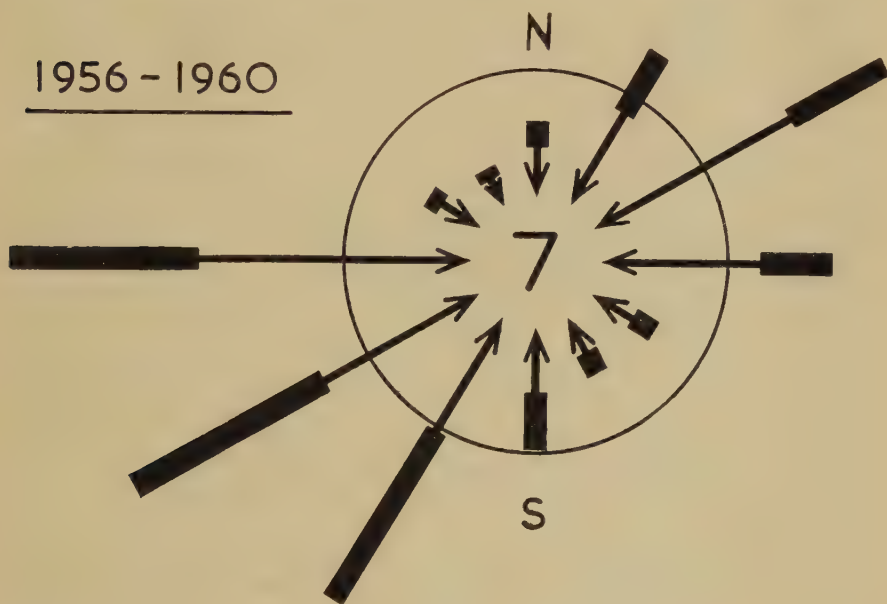


Fig. 1. Wind Rose showing the percentage frequency of winds blowing from twelve points of the compass at Long Ashton from 1956-1960. *Wind Direction.* Frequency shown by length of arrows, which fly with wind ; distance from head of arrow to circle=5%. *Wind Speed.* Shown by thickness of arrow ; thin portion=1-7 m.p.h. ; thick portion>7 m.p.h. *Calms.* Percentage given by figure in centre of circle.

Wind Direction.

The general pattern of the rose is similar to that at most anemometer stations in England, where the winds most often experienced are those blowing from the SW and W with a secondary maximum from a NE point. The difference between the frequency of winds from the prevailing directions and that from the more sheltered ones is, however, more marked than usual. While this may in some degree be due to the effect of the nearby Bristol Channel,

which funnels Atlantic winds into the Severn estuary, the effects of local topography play a major part. The hill along the north side of the valley provides considerable shelter while, to the south, the gentler slopes which eventually rise to the hills of Mendip, some ten miles distant, combine to canalise the winds along the valley. Compared with frequencies of 18% from the W; 15% from the WSW and 13% from the NE, the frequency from the more sheltered directions such as NW and SE is under 3%.

The predominance of winds from W and SW is most marked during June, July and August, though occasionally there are marked exceptions such as 1955, when 52% of the winds in July and August were from a NE point.

North-easterly winds are most common early in the year, when the weather is often dominated by anticyclones over northern Europe. In 1953, 54% of January's winds were from the NE, and in 1959, 45% of the winds in February were from the same point.

Wind Speed.

Calms and light winds up to three m.p.h. are least frequent in spring and early summer, and rise to a maximum in early winter. The second half of 1955 was notable for a long period of light winds and calms, only 55% of the winds exceeding 3 m.p.h. In October, 1953, only 31% of the winds exceeded this speed.

The strength of the wind is of particular importance to fruitgrowers at spraying and blossoming times, and in late summer when strong winds may blow the fruits from the trees. Gales strong enough to uproot established trees are fortunately rare at Long Ashton.

Calms and winds of up to 7 m.p.h. occupied 54% of the time from 1956 to 1960. Winds averaging this speed are accompanied by gusts of 10-15 m.p.h., and are at the upper limit of the range of speed within which spraying may be carried out without excessive hindrance. Winds are on the average stronger during the day than at night. For example, from April to June, the busiest spraying quarter of the year, light winds and calms amounted to 72% of the twelve hours from 18-00 G.M.T., compared with 45% for the twelve daytime hours from 06-00 G.M.T., the range of the latter being from 36% in 1958 to 57 % in 1960.

From July to August, winds with an average speed of 15 m.p.h. or more, accompanied by gusts rising to about twice the mean speed, occur on average on between three and four days per month. Under these conditions damage may be caused through fruit being blown from the trees. One of the strongest winds recorded during this season was on the night of September 21/22, 1953; during the peak hour this reached an average speed of 26 m.p.h., accompanied by a gust of 62 m.p.h. Again, on August 18, 1956, there was a wind of 25 m.p.h., with gusts up to 57 m.p.h.

The standard height to which measurements of wind speed are referred is 33' above ground (1). As the height of the mast at Long Ashton is 9' less than this, when compiling Table I a correction of +20% was made to speeds indicated by the anemograph, in order that the observations might be compared with estimates made according to the Beaufort scale. The table shows the number of days in each month on which the winds averaged 25 m.p.h. (Beaufort Force 6) or more for at least one hour throughout the period 1947 to 1960.

TABLE I.
NUMBER OF DAYS WITH WINDS OF 25 M.P.H. AND UPWARDS.
Total for 1947-1960.

Month	Source of Winds				Total
	NW & N	NE & E	SE & S	SW & W	
January	0	3	2	30	35
February	0	0	3	20	23
March	0	4	5	24	33
April	0	0	0	28	28
May	0	1	0	4	5
June	0	0	0	3	3
July	0	0	0	5	5
August	0	0	0	8	8
September	0	0	0	5	5
October	0	1	0	3	4
November	0	0	0	19	19
December	0	1	2	31	34
Total	0	10	12	180	202

It will be seen that winds of Force 6 or upwards have on the average occurred on 14 days in the year; the extremes are represented by 1959, in which there were only 4 days with winds of this speed, and 1948, in which there were 34. They are most frequent from December to April. Nearly all these winds blow from SW or W, the few from between NE and SE occurring mainly in the early part of the year. The strongest NE wind registered during the fourteen years was on March 29, 1952, when the highest hourly mean speed was 33 m.p.h. (Force 7). Strong winds are seldom recorded on more than five days per month during the winter, or on more than two in the period April to November, though April, 1948, was notable for strong winds on ten days during the month.

Gales—in which the wind reaches an average speed of at least 39 m.p.h. (Force 8) or over for at least ten minutes during the day—are uncommon, and have been recorded on only 23 days. Usually they occur during squalls and are of short duration, often lasting less than thirty minutes. The most severe and prolonged gale during the period under review, and probably for many years previously, occurred on March 16, 1947, when the wind from between W and SW averaged Force 8 for three hours, becoming Force 9 (53 m.p.h.) for a time, with a maximum gust of 78 m.p.h. A gust of 72 m.p.h. was recorded on November 27, 1954, the only other occasion when 70 m.p.h. was exceeded, though between 1925 and 1947 there are at least two reliable records of gusts exceeding 70 m.p.h. Gusts of 60 m.p.h. or more occur up to three times a year, and have been recorded in all months except May, June and July, though there is only one instance of such a gust in August.

REFERENCE.

- (1) ANON. *Observer's Handbook* (1956). Air Ministry; M.O. 554 (2nd Edn.), H.M.S.O.

METEOROLOGICAL RECORDS, 1960.

Month	AIR TEMPERATURE (°F.)						RAINFALL (")			SUNSHINE (hours)		SOIL TEMPERATURE at 09-00 G.M.T.		
	Means		Diff. from normal	Max.	Min.	No. of ground frosts	Total	Per cent of average	Most in a day	Daily mean	Per cent of average	4"	8"	24"
	A Max.	B Min.												
January	45.8	36.0	+0.5	55	24	16	3.57	107	0.98	1.06	68	39.4	40.3	43.1
February	45.9	35.2	-0.2	61	25	18	2.56	99	0.37	2.71	115	38.3	39.9	41.8
March	50.2	38.5	+0.6	61	31	11	2.53	117	0.47	2.50	64	42.5	43.2	44.9
April	56.8	40.5	+1.1	64	29	10	1.50	68	0.53	5.24	95	48.2	47.9	48.2
May	64.5	47.7	+3.0	74	37	1	1.16	45	0.40	6.04	96	57.5	56.4	55.7
June	70.2	52.7	+3.0	81	44	0	2.21	100	0.56	7.93	113	64.9	63.8	62.3
July	66.3	53.2	-1.8	72	45	0	4.86	155	0.94	4.97	78	63.1	62.1	62.5
August	66.7	51.7	-1.8	73	43	0	4.83	132	1.01	5.17	87	61.5	61.2	62.2
September	62.8	49.3	-1.0	73	40	1	4.91	151	1.16	3.87	83	57.5	58.1	60.2
October	56.9	45.4	0.0	65	31	7	6.34	163	1.14	2.28	69	51.9	52.5	55.2
November	52.0	40.8	+1.6	61	28	12	4.80	130	0.74	2.60	138	45.7	46.4	49.3
December	45.3	35.8	-0.9	54	28	23	4.86	133	1.91	1.83	124	40.7	41.9	45.1
Totals or Means	56.9	43.9	+0.3			99	44.13	122		3.84	92	50.1	51.1	52.5

G. E. CLOTHIER.

THE JOURNAL OF HORTICULTURAL SCIENCE

The Journal of Horticultural Science, which is sponsored by the Long Ashton Research Station and the East Malling Research Station, welcomes contributions on horticultural science from Research Institutes at home and abroad. Papers for publication and all communications should be sent to the Editors at East Malling Research Station, Maidstone, Kent.

Papers in the current *Volume* 36 (1961) come from the Sponsor Stations, the Glasshouse Crops Research Institute, the Scottish Horticultural Research Institute, the John Innes Institution, the National Vegetable Research Station, Manchester University and Wye College, and from Canada, New Zealand and Ghana. Fruit crops reported on are apple, plum, raspberry, strawberry and cocoa ; there is a first report of investigations on rootstocks for roses ; tomatoes, mushrooms and garden beet are the subjects of other papers, while there is also one on the nutrition of a range of vegetable crops ; an investigation on the soil for John Innes composts is reported. Several physiological studies are described, involving such subjects as flower initiation, influence of environment, storage, and of shade and manuring on cocoa ; other papers describe work on root anatomy, plant breeding, manuring for seed production, frost resistance, fruit tree viruses and the apple spray programme ; there is an account of a long term investigation on rootstocks for plums.

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